INDUCED MUTATION IN SWEET POTATO AIMED AT IMPROVED QUALITY AND DROUGHT ADAPTATION

by

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DECLARATION

"I declare that the dissertation hereby submitted by me for the **Magistaire Scientiae Agriculturae** degree at the University of the Free State is my own independent work and has not previously been submitted by me at another university/faculty. I furthermore cede copyright of the dissertation in favour of the University of the Free State."

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Mmapaseka Elizabeth Malebana Date

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LIST OF ABBREVIATIONS

ARC-VOPI	Agricultural Research Council – Vegetable and Ornamental Plants Institute
β-carotene	Beta carotene
^{60}Co	Cobalt 60
CIP	International Potato Center
CRD	Complete Randomised Design
CSPI	Centre for Science in the Public Interest
CV	Coefficient of variation
DTD	Days to death
EMS	Ethyl methanesulfonate
HPLC	High performance liquid chromatography
LAN	Limestone Ammonium Nitrate
LD_{30}	30% Lethal dosage
LD_{50}	50% Lethal dosage
LSD	Least significant difference
Min	Minutes
MS	Murashige and Skoog
MT	Metric Ton
NS	Non significant
OFSP	Orange-fleshed sweet potato
PEG	Polyethylene glycol
PPM™	Plant Preservative Mixture
PYT	Preliminary yield trial
RCBD	Randomised Complete Block Design
RDA	Recommended daily allowance
SA	South Africa
SANBS	South African National Blood Service
SPFMV	Sweet potato feathery mottle virus
SSA	Sub Saharan Africa
UFS	University of the Free State
VAD	Vitamin A deficiency

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CHAPTER 1
GENERAL INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam) is regarded as one of the major staple food crops feeding millions of people worldwide with a wide adaptation to various environmental conditions (Kays 2005; Lebot 2009). The world production figures were estimated at 106.50 million metric tons (MT) with China as the largest producer at 80.50 million MT followed by Nigeria as the largest producer in Africa at 3.31 million MT (FAOSTAT 2011). Following on cassava and yam, sweet potato is the third most important tuber/root crop produced in sub-Saharan Africa (SSA) hence making sweet potato a potential food security crop. In SA sweet potato is popular amongst resource poor farmers and rural communities and is mainly produced under rain-fed conditions (Laurie *et al.* 2004). Sweet potato production is in many ways ideal for these rural communities and resource poor farmers as it fits in with low input agriculture (Laurie *et al.* 2009a). Some of the advantages are that the crop produces acceptable yields in soils with low fertility; it is more drought tolerant than other conventional vegetable crops, it crowds out weeds quickly and is susceptible to relatively few pests (Woolfe 1992; Laurie *et al.* 2009a).

Sub-Saharan Africa is said to be the most food insecure region in the world (Orindi 2009; FAO 2012) and predictions indicate that by 2020 yields could be reduced by up to 50% in rain-fed agricultural systems, thus increasing poverty and food insecurity (Orindi 2009). The alarming increase of human population in developing countries and the unstable economic status have also contributed to increased food shortages (Ishida *et al.* 2000; Tonukari and Omotor 2010; Saltzman *et al.* 2013). Furthermore, climate change has posed a serious threat to agricultural production because of the change in rainfall distribution that has resulted in prolonged dry periods and elevated temperatures (Ishida *et al.* 2000). As a result, food security status has declined, especially in developing countries. South Africa (SA) is classified as a water-stressed country (Bennie and Hensley 2001) and although sweet potato is described as a hardy crop with some drought tolerance, the current water stress conditions have negative effects on production under rain-fed conditions (Laurie *et al.* 2009b). To reduce the impact of climate change on food security and poverty, it is essential for farmers to adopt agricultural initiatives that promote the development and cultivation of improved drought tolerant varieties (Hamdy *et al.* 2003; Tonukari and Omotor 2010) for sustainable food production. Drought tolerance is therefore an important trait to include in cultivar improvement initiatives.

Micronutrient deficiency has been a major health concern in developing countries, being directly responsible for conditions such as xeropthalmia associated with vitamin A deficiency (VAD) and anaemia caused by iron deficiency (Hillocks 2011). Other nutrients listed as the most lacking in human diets are zinc, copper, calcium and magnesium (White and Broadley 2009). Health interventions in most developing countries have introduced vitamin and mineral supplements as an approach to address micronutrient deficiency. However, these supplements are usually imported and therefore not sustainable due to high costs involved (Hillocks 2011). Crop-based improvement approaches like biofortification are considered to be cost effective, sustainable, long term supplementary approaches that could help meet the nutritional needs of rural populations in developing countries (Hillocks 2011; Saltzman *et al.* 2013). Biofortification is defined as "the development of micronutrient staple crops using the best traditional breeding practices and modern technology" (Nestel *et al.* 2006). Saltzman and colleagues (2013) define the process of biofortification as "breeding nutrients into food crops". One example of biofortification is breeding and selection of orange-fleshed sweet potato (OFSP) varieties that has become one of the important projects coordinated by the International Potato Center (CIP) and HarvestPlus to control VAD in developing countries (HarvestPlus 2004) including SA. Orange fleshed sweet potato is currently promoted internationally as a biofortified food that provides considerable amounts of pro-vitamin A (Burri 2011), much higher than that of Swiss chard and pumpkin; and slightly lower than that of carrot (Wolmarans *et al*. 2010).

Sweet potato needs to be improved genetically so as to increase its impact as a food security crop as well as its contribution to addressing nutrient deficiency. To maximise sweet potato utilisation and acceptance by consumers, improved varieties with a combination of good yield, increased micronutrient content, high dry mass content and improved drought tolerance are required (Tumwegamire *et al*. 2004; Laurie *et al.* 2009a; Laurie 2010). Traditional plant breeding has been remarkably successful in creating improved varieties for different crops, but genetic complications in sweet potato such as poor flower induction, low seed set and incompatibility (du Plooy 1986; Broertjies and van Hartem 1988; Kanju 2000) have slowed the crop's genetic improvement progress. Genetic complications encountered in conventional breeding have motivated breeders to use induced mutations in their crop improvement initiatives for both seed and vegetatively propagated crops. The success of induced mutations as a breeding tool has resulted in more than 3 000 mutant varieties developed worldwide in food crops (IAEA 2013).

Induced mutations was used in this study as a breeding tool to generate sweet potato mutant germplasm with enhanced yield, ability to withstand prolonged water stress conditions and increased nutritional quality. Developed mutant lines will be included in the South African sweet potato breeding programme to improve food security and alleviate nutrient deficiency.

Research aim and objectives

This study aimed at applying mutagenesis in the form of gamma irradiation as a breeding tool to induce mutations in high yielding, acceptable and widely adapted, local cream-fleshed sweet potato varieties. The effects of gamma irradiation on root yield, nutritional quality and drought tolerance were investigated.

The specific objectives of the study were:

1. To determine optimal dosages of gamma rays to induce mutations in selected varieties through *in vitro* radio sensitivity tests.
2. To generate a sweet potato mutant population and identify putative mutants with morphological changes and improved drought tolerance after gamma irradiation.
3. To identify putative mutants with improved agronomic traits (such as root yield and dry mass content) and increased nutritional value, for further evaluation in the breeding programme.

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CHAPTER 2
INDUCED MUTATION AS A BREEDING TOOL IN SWEET POTATO IMPROVEMENT INITIATITIVES – REVIEW

2.1 SWEET POTATO

Sweet potato (*Ipomoea batatas*) is a member of the Convolvulaceae family and the only natural hexaploid plant of the morning glory family that has 90 chromosomes (Hall and Phatak 1993). Although the crop originated in Central America, its wide adaptation has led to its successful introduction and production in more than 166 countries worldwide (Vimala *et al.* 2011). Sweet potato is one of the seven major staple crops in the world (FAOSTAT 2011) grown for different purposes by different countries. There is a wide range of cultivars available, offering great potential for different types of utilisation. The available cultivars differ in flesh colour, dry matter content, starch and sugar contents (Low *et al.* 2009). Sweet potato is grown in Africa predominantly for human consumption, while in China it is mainly grown for bio-ethanol production (International Life Sciences Institute 2008). Sweet potato consumption also differs between and within countries by provinces/regions and by income groups. Storage roots can be eaten baked, cooked or eaten raw and can also be processed into jam, juice, chips and other nutritious foods (Laurie *et al.* 2004). Sweet potato is also used to make breads, cakes and candies (Woolfe 1992). Tips of shoots and young leaves can be eaten as a leafy vegetable; while vines and crop residue provide nutritious feed for animals (Wambugu 2003) making the whole plant useful.

2.1.1 Agricultural and economic importance

Sweet potato is one of the traditional crops that requires relatively low inputs (Jain 2005; Laurie *et al.* 2009a), thus significantly contributing to sustainable agricultural production and increasing its potential as a food security crop. The crop is known for its ability to adapt to a wide range of habitats (Aina *et al.* 2009) and to grow in soils with low fertility, but still produce acceptable yields (Lebot 2009). For example, the crop is adapted to all nine provinces of SA and can be grown under water stress conditions, provided enough water is supplied during the first two weeks after planting (Laurie *et al.* 2009a). The adaptability and some level of drought tolerance in sweet potato is significant to agricultural production in SA (Alleman *et al.* 2004) because agricultural systems in the country are developed mainly under arid and semi-arid conditions (Bennie and Hensley 2001).

Sweet potato remains underutilised in SA, resulting in low consumption and ultimately low production figures when compared to that of other African countries (Low *et al.* 2009; FAOSTAT 2011). Urbanisation is one of the main reasons causing a decrease in the consumption of fresh sweet potato roots in SSA due to price, convenience and status (Low *et al.* 2009). The Department of Agriculture, Forestry and Fisheries (2012) estimated sweet potato production in SA at 63 000 MT for the 2011/2012 season with 22 000 MT sold on the major fresh produce markets. The current low figures for sales on the national fresh produce markets is due to the fact that resource poor farmers and rural communities traditionally plant sweet potato as a food security crop and/or cash crop that is mainly sold in street markets. Although the sweet potato industry is considerably smaller than that of potato in the country, the price at the fresh produce markets remained higher than that of potatoes during the 2011/2012 season (Department of Agriculture, Forestry and Fisheries 2012) highlighting the crop's economic value. Further genetic improvement of sweet potato could increase the crop's potential in agricultural production systems and improve its economic status in developing countries, especially SA.

2.1.2 Nutritional value

Sweet potato has excellent edible energy and protein production efficiency in the developing world as it heads a list of eight important developing world crops in terms of quantity of energy produced per hectare per day (Woolfe 1992). The Centre for Science in the Public Interest (CSPI) also classified sweet potato as the most nutritious vegetable (Ehler 2010), hence its nutritional importance. Together with wide genetic variability and adaptability, sweet potato is also known for its extensive phenotypic variation in skin colour, flesh colour, root shape and size. Root flesh colour varies from white, yellow, orange to purple, which reflects variation in nutrient concentrations (Vimala *et al.* 2011; Leksrisompong *et al.* 2012). The OFSP types in particular, are a good source of beta-carotene (β-carotene) which is a precursor of vitamin A, dietary fibre and minerals (Nestel *et al.* 2006; Bengtsson *et al.* 2009; Burri 2011) and the purple flesh types are rich in anthocyanins and phenolic compounds (Leksrisompong *et al.* 2012).

Beta-carotene is the most important pro-vitamin A carotenoid and the predominant carotenoid found in OFSP (Bengtsson *et al.* 2009; Low *et al.* 2009; Wolmarans *et al.* 2010; Burri 2011). The consumption of OFSP as staple food can supply significant amounts of vitamin A and energy, thus addressing both vitamin A and malnutrition (Low *et al.* 2009). Laurie and co-workers (2012) calculated that daily feeding of 4-8 year old children with an average portion of 125 g OFSP provides more than 100% of the

recommended daily allowance (RDA) of vitamin A required. Furthermore, if dark orange varieties like Resisto (an imported USA dark orange variety) are used, a quarter portion of ± 32 g would still provide the amounts required. Roots of OFSP varieties promoted in SA contains 5091 to 16456 µg 100 g^{-1} trans-β-carotene (Laurie *et al.* 2012)

Although the consumption of sweet potato leaves is more limited in SA (Alleman *et al.* 2004), both the storage roots and fresh leaves are nutritious and important in human health. A recent study conducted on sweet potato suggested that the consumption of fresh leaves for longer than 14 days could result in reductions in blood pressure and body weight (Johnson and Pace 2010). Sweet potato leaves are rich in vitamins, minerals, antioxidants and dietary fibre (Wambugu 2003; Johnson and Pace 2010) and daily consumption could reduce cardiovascular disease risk (Johnson and Pace 2010). The nutritional value of sweet potato leaves was earlier reported in a study to assess the pro-vitamin A content and sensory attributes of new sweet potato genotypes in Ghana (Ofori *et al.* 2009). Leaf and root samples were analysed and it was found that β-carotene was the dominant carotenoid in fresh leaves as it is in OFSP storage roots, ranging from 508 to 3860 µg 100 g^{-1}. These contents were lower than those found in spinach leaves, but were still significant for human nutrition. The findings concluded that, for the clones used in the study, the fresh sweet potato leaves were a richer source of pro-vitamin A than the fresh roots, however varieties with high pro-vitamin A content in the fresh leaves, had low contents in the fresh storage roots (Ofori *et al.* 2009). The reported nutritional contents of both sweet potato leaves and storage roots, highlight the crop's potential and significance in addressing nutrient deficiency in developing countries.

2.2 IMPORTANT SWEET POTATO TRAITS FOR SELECTION IN BREEDING AND TECHNIQUES USED

2.2.1 Breeding objectives

Formal sweet potato breeding in SA was initiated in 1952 with the aim of developing high yielding and adapted cream-fleshed varieties for the commercial market (Bester and Louw 1992). The programme released new cultivars that drive the sweet potato industry in SA. Twelve cream-fleshed cultivars were released between 1952 and 1989 (Bester and Louw 1992); seven more were released between 2003 and 2004, which focused on the needs of resource poor farmers (Laurie and Magoro 2008). Recently six OFSP varieties were released for food-based programmes in SA and for the export market (Laurie *et al.* 2009a, Laurie 2010). The development of OFSP varieties was initiated in

SA in the late 1990's and became the main focus of the breeding programme in 2003 (Laurie *et al.* 2009a). The aim is to develop improved sweet potato varieties with good yield (± 30 t ha^{-1}), good storage root quality (smooth and firm, with uniform shape and size, free from mechanical damage with uniform peel colour typical of the variety); sweet taste, dry texture (> 25%); and high β-carotene content (> 7000 µg 100 g^{-1}) through conventional breeding (Laurie *et al.* 2009a; Laurie 2010).

Important additional traits in the breeding programme include drought and virus tolerance and tolerance to *Alternaria* leaf and stem blight. In terms of available water SA is the 30th driest country in the world (Schreiner *et al.* 2010) hence drought tolerance has become an important trait in crop improvement to secure food production. Virus infection is the main disease limiting sweet potato production worldwide (Salazar and Fuentes 2001) and the major infection in SA is by sweet potato feathery mottle virus (SPFMV) which can result in up to 80% yield loss in susceptible varieties (Domola *et al.* 2008). *Alternaria* stem blight has also become an important trait in the sweet potato breeding programme because of the crop's sensitivity to infection (Osiru *et al.* 2007; Thompson et al. 2011).

2.2.2 Selection methods

The breeding programme in SA has successfully released new improved varieties through conventional breeding. Desirable characteristics from selected parents were combined through the polycross method followed by clonal selection and multi-location trials as illustrated in Fig. 2.1 (Laurie *et al.* 2009a). The programme also included the development of new improved progenies through direct crossing between female and male parents selected for specific desirable traits in a crossing block. The selection process starts with single plant selections based on the storage root size, quality and flesh colour; then the selected clones are further evaluated in preliminary and intermediate yield trials; and ultimately evaluation is done across locations in the advanced yield trials to determine adaptability and stability of genotypes (Laurie *et al.* 2009a; 2010).

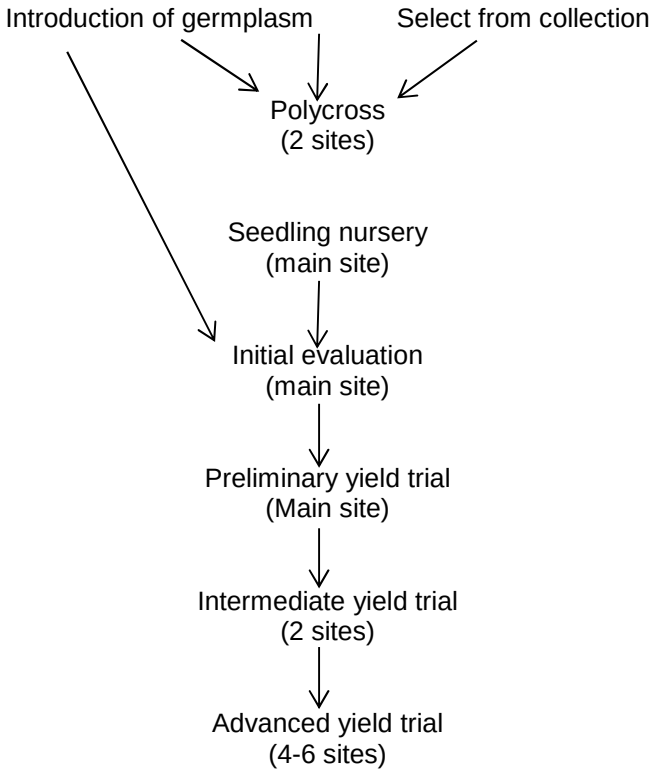

Fig. 2.1 Schematic presentation of the OFSP breeding programme in SA as adapted from Laurie *et al.* (2009a)

The important agronomic traits to consider during field evaluation are yield, quality, disease tolerance and morphological characterisation of each genotype. At harvest, genotypes are evaluated on storage root yield (marketable and unmarketable root yield); flesh colour; shape and size; raw and cooked taste; total soluble solids measured with a refractometer; and dry mass content (Abidin *et al.* 2005; Laurie *et al.* 2009a; Osiru *et al.* 2007). Evaluation of yield involves testing of varieties in different locations over a period of years and the genotype and environment interaction knowledge can assist the breeder in determining yield stability (Hall and Phatak 1993; Abidin *et al.* 2005).

It is important to have simple screening techniques to use during the early stages of progeny selection for important nutrients, because nutrient analyses can be costly (Lebot *et al.* 2011). Therefore quick screening methods are used for the selection of orange fleshed lines and for drought tolerance separately in the breeding programme. Orange-fleshed seedlings are identified two to three months after planting in a glass house or on a seedbed by sectioning the thickened roots. This is an easy method and shortens the period to identify orange flesh colour by two months as well as saving cost on large field evaluation trials (Laurie *et al.* 2009a). In later generations colour measurements can be employed as indication of β-carotene content (Takahata *et al.* 1993; Laurie 2010). The total carotenoid content can be determined by spectrophotometry and β-carotene

content by high performance liquid chromatography (HPLC) as previously described by Low and van Jaarsveld (2008). Drought tolerance screening, as adapted from Singh *et al.* (1999) is used routinely at Agicultural Research Council – Vegetable and Ornamental Plants Institute (ARC-VOPI) to screen breeding lines for drought tolerance. The methods involve planting in plastic boxes and screening for drought tolerance at the early vegetative stage based on survival rate after inducing drought stress as well as days to permanent wilting (Laurie *et al.* 2009b).

2.2.3 Limitations in the conventional sweet potato breeding programme

Although there is wide genetic variation to be exploited, breeders worldwide agrees that sweet potato is a complicated crop to breed using conventional or traditional breeding methods because of poor flowering. If the plants get to flower, seed set is low due to incompatibility barriers within the crop (du Plooy 1986; Broertjies and van Hartem 1988; Kanju 2000). Poor flowering was also identified as the main complication encountered when combining high dry mass content with other desired traits in the South African breeding programme because other clones did not flower (Laurie *et al.* 2009a), making it difficult to combine some desirable traits that could contribute to the crop's genetic improvement.

The South African breeding programme has managed to release good varieties with average yields and high β-carotene content through the conventional breeding methods (Laurie 2010). However, the released OFSP varieties are characterised by slightly lower yield and lower dry mass content as compared to the common cream-fleshed varieties (Laurie 2010). Again the tested OFSP varieties do not provide adequate dietary requirements for other minerals like zinc, iron, magnesium, calcium and phosphates (Laurie *et al.* 2012) hence the need to increase the availability of these micronutrients in order to alleviate nutrient deficiency as well as to improve drought tolerance in adapted varieties for increased sustainable yields.

2.3 FOOD SECURITY AND NUTRIENT DEFICIENCY

Food security and nutrition are of critical importance in developing countries (Gruissem 2010) hence the millions of dollars spent annually by governments on food aid programmes to alleviate hunger and poverty (Labadarios *et al.* 2011). There are many definitions given for food security, but all point to "availability of enough food always for everyone to eat". Food security is defined as "the state when all people in the society have enough food at all times for an active, healthy lifestyle" (Labadarios *et al.* 2011).

FAO (2012) reported positive global progress in reducing hunger between the years 1990-2 and 2010-2 but also reported an increase in hunger and poverty from 17% to 27% in SSA. In SA, Labadarios and co-workers (2011) reported an overall decrease in food insecurity from 52.3% to 25.9% for both urban and rural populations between 1999 and 2009. However, the high number of refugees coming into SA and the food insecurity status reported in SSA (FAO 2012) could result in food shortages and ultimately have a negative influence on the food security status in the country.

2.3.1 Effect of limited water availability on food security

The ever increasing human population leads to an increase in food demand (Tonukari and Omotor 2010) and that exerts pressure on the available agricultural resources. Kamara and Sally (2004) tested the link between population growth, water availability and food insecurity in SA. They realised that with the population increase, water availability is declining and this leads to reduced total food production as a result of limited irrigation water. On the other hand, climate change has resulted in erratic rainfall patterns as well as severe drought conditions that threaten sustainable crop production in developing countries (Orindi 2009).

Agricultural growth is one of the important solutions in reducing hunger and poverty (FAO 2012; Tonukari and Omotor 2010) especially in rural areas where agriculture is the main source of income and employment (Naylor *et al.* 2004). The adoption of agricultural practices that ensure rainfall utilisation for dryland production is essential (Bennie and Hensley 2001) and drought stress can be alleviated by using adapted genotypes with drought tolerance (Hamdy *et al.* 2003). The prevailing dry conditions have led to production threats even for crops with inherent drought tolerance (Laurie *et al.* 2009b). Sweet potato is a potential food security crop with the ability to produce acceptable yields even under occasional dry spells (Laurie *et al.* 2009b; Low *et al.* 2009), but the current prolonged water stress conditions during the growing period seem to negatively affect yields under rain-fed conditions (Ekanayake *et al.* 1988; van Heerden and Laurie 2008) resulting in reduced food production. New crop varieties with improved drought tolerance are essential to produce sustainable good yields under the current limited water resources.

2.3.2 Nutrient deficiency

Food security does not only imply an increase in the quantity of energy intake, but also includes the improvement of food in terms of dietary diversity and nutrient content (FAO 2012). Malnutrition is generally described as a medical condition caused by improper or

inadequate uptake of nutrients in the human body (Mashaba and Barros 2011) and the FAO (2012) reported that 870 million people worldwide are chronically undernourished. Although Labadarios and co-workers (2011) reported an increase in food security, they also found that in poorer households children were fed a poor diet and that in 2005 18% of the children were chronically stunted as a result of malnutrition within these families.

Micronutrient deficiency affects human health (Hillocks 2011) and the important micronutrients found to be deficient in the diets of poor populations in the developing countries are vitamin A, iron and zinc (White and Broadley 2009). It is said that about 11% of deaths occurring before the age of 5 in developing countries, could be related to vitamin A, zinc and iodine deficiency (Murgia *et al.* 2012). Vitamin A is deficient in the diet of many rural people in SA due to poverty (Labadarios *et al.* 2007) and the deficiency reduces the ability of the body to fight against infection resulting in increased susceptibility to childhood infections like measles and diarrhoea, and ultimately leading to death (Mukherjee and Ilangantileke 2001). Iron deficiency leads to increased vulnerability to infection, impaired growth and cognitive function and may lead to disability in children under 5 years of age (Leyva-Guerrero *et al.* 2012). Another deficient mineral is magnesium, which is important for circulatory diseases and calcium metabolism in bones (Ishida *et al.* 2000).

Based on the overall nutritional composition reported in literature, it is evident that sweet potato is a highly nutritious crop that has great potential to address nutrient deficiency in developing countries (Woolfe 1992; Wambugu 2003; Nestel *et al.* 2006; Bengtsson *et al.* 2009; Laurie *et al.* 2009a; Ofori *et al.* 2009; Ehler 2010; Wolmarans *et al.* 2010; Burri 2011; Leksrisompong *et al.* 2012). With the high β-carotene content found in OFSP, there are moderate levels of iron and zinc (Low *et al.* 2009) and increasing the availability of these micronutrients in sweet potato might significantly improve the potential as well as the consumption of the crop in developing countries.

2.4 BIOFORTIFICATION AND BIOTECHNOLOGY

To assist the governments to achieve their main strategic goal of addressing nutrient deficiency (Nestel *et al.* 2006) crop improvement approaches that breed nutrients into crops (Saltzman *et al.* 2013) are necessary. These approaches are sustainable in a sense that nutritional needs of populations worldwide, especially in developing countries, would be met in a cost-effective manner (Nestel *et al.* 2006; Akram *et al.* 2011; Hillocks 2011; Saltzman *et al.* 2013) and the improved varieties will always be available for the

poor communities to grow and consume even when the governments have shifted the focus from nutrient deficiency (Nestel *et al.* 2006).

Jain and Suprassanna (2011) highlighted that the main objective for crop breeders worldwide is to sustain food production and improve nutrition, hence the need for breeders to include biofortification as one of their key objectives in addition to the on-going breeding objectives of high yield, disease tolerance and good quality (Nestel *et al.* 2006; Hillocks 2011). Biofortification of staple crops is essential because it targets low income households in which staple food is the predominant diet (Nestel *et al.* 2006) and one good example of such enriched products is vitamin A rich OFSP (HarvestPlus 2004). Frequent feeding of OFSP can ensure adequate intake of the RDA of vitamin A (Woolfe 1992; Mukherjee and Ilangantileke 2001; Laurie *et al.* 2012) and moreover, sweet potatoes are affordable and easily available even for the rural communities.

Modern breeding concerns creating variation and this can be done by combining several breeding techniques (Ahloowalia and Maluszynski 2001). The combination of conventional and biotechnology methods can be used to exploit genes for essential nutrients (Johns and Eyzaguirre 2007) and biotechnology tools have so far created new opportunities in the improvement and availability of the total amount of nutrients in food crops worldwide (Jain and Suprasanna 2011). These tools are available to optimise the quality of food so as to ensure food security and meet the nutritional needs of rural communities in developing countries (Tonukari and Omotor 2010; Jain and Suprasanna 2011).

Biotechnology refers to a set of medical, agricultural and industrial techniques that use living organisms to create new or improved products and processes (Johns and Eyzaguirre 2007). Different biotechnology techniques including gene modification, have been proposed and applied for biofortification of staple foods worldwide (Tonukari and Omotor 2010). The transgenic golden rice with β-carotene content of ±3100 µg 100 g^{-1} (Nestel *et al.* 2006) was developed through the genetic engineering approach and is available to consumers as part of addressing nutrient deficiency (Jain and Suprasanna 2011). Biotechnology is also available for breeders of vegetatively propagated crops like sweet potato in which seed production is limited, thus making conventional breeding techniques complicated (Alleman *et al.* 2004). Some of the options available to the plant breeder are the use of *in vitro* culture for rapid multiplication, molecular markers to select genotypes with specific traits and mutagenesis as a tool to induce mutation (Ahloowalia and Maluszynski 2001).

2.5 INDUCED MUTATION BREEDING

Mutation is defined as “heritable change to the genetic make-up of an individual that occurs naturally in plants” (Mba *et al.* 2009). Spontaneous mutations occur naturally in vegetatively propagated crops and these mutations have been the single most contributing factor in evolution as the changes that are passed on to the offspring lead to the development of new individuals/varieties (Brunner 1995; Mba *et al.* 2010). However, the rate of spontaneous mutations is low and cannot always be exploited for breeding, thus the need for artificial mutations in the form of induced mutations or mutagenesis (Jain and Suprasanna 2011).

It is a fact that to develop new varieties, genetic variability is desirable and this can be artificially created by inducing mutation through mutagen treatments (Jain and Suprasanna 2011). Mutagenesis has become an important crop improvement tool available to breeders with no regulatory restrictions imposed as with genetically modified crops (Parry *et al.* 2009) and mutant varieties are readily accepted by consumers (Jain and Suprasanna 2011). Mutagenesis in conjunction with conventional breeding methods could result in mutant varieties with desirable traits (Jain and Suprasanna 2011) including enhanced nutritional quality, increased yields and drought adaptation.

2.5.1 Principles of induced mutation

Induced mutation is aimed at optimising genetic variation by creating mutagenesis through altering one or two major traits, while maintaining the major genetic composition of the variety (Ahloowalia *et al*. 2004; Owoseni *et al.* 2006; Babaei *et al.* 2010). Mutations are artificially induced by exposing plant material to mutagenic treatments to broaden the genetic base of germplasm for plant breeding (Mba *et al.* 2010). Mutation occurs when the mutagen treatment applied breaks the nuclear DNA and during the process of DNA repair, mutations occur randomly and are heritable (Jain and Suprasanna 2011). The three main effects of mutagenesis are point mutations, physiological damage and chromosomal aberrations. Induced mutagenesis generates allelic variants of genes that modulate the expression of traits (Mba *et al.* 2009)

Conventional breeding techniques rely on combining the available genetic variation through hybridisation, which is usually impractical in vegetatively propagated crops (Mba *et al.* 2009) because of sexual incompatibility barriers. The difference between induced mutations and conventional breeding is that the latter involves the production of new genetic combinations from already existing parental genes, while mutagen treatments

can create new gene combinations with high mutation frequency (Majeed *et al.* 2010). Induced mutations are useful in creating variability that is not available in the gene pool or to correct a specific deficiency of an otherwise outstanding genotype (Kumar and Pandey 2008). The altered trait can also cause a synergistic effect on the cultivation of the crop, for example, the short height genotypes in maize and rice that have contributed significantly to increasing grain yield because of their tolerance to lodging and high planting density (Ahloowalia *et al.* 2004).

2.5.2 Mutagen treatment and suitable applications to induce mutation

The type of irradiation and dosage is critical as it influences the success of mutation induction and plant regeneration (Ahloowalia and Maluszynski 2001; Owoseni *et al.* 2006). The mutation breeder has a choice of selecting from physical (for example X-rays and gamma rays) and chemical mutagens (such as ethyl methanesulfonate – EMS and sodium azide) available to induce mutations in plant material (Velmurugan *et al.* 2010).

The selection of effective mutagens is essential in obtaining efficient and desirable mutations (Solanki and Sharma 1994). Combining both physical and chemical mutagens may increase the frequency of main mutations leading to economically useful characters when compared to single treatments (Mehandjiev *et al.* 2001). The benefits of physical mutagens are listed as accurate dosimetry and reasonable reproducibility, whereas chemical mutagens offer high mutation rate and predominantly point mutations (Jain 2005). Among the available physical mutagens, gamma irradiation has been widely used for the development of useful mutants in both seed and vegetatively propagated crops (Brunner 1995; Kharkwal and Shu 2009; Jain and Suprasanna 2011). More than 60% of the released mutant varieties worldwide are from gamma rays, 22% from X-rays and the rest were induced through other treatments including chemical mutagens (Ahloowalia *et al.* 2004; Shu and Lagoda 2007).

Gamma rays have proven to be useful in inducing variability and increasing mutation frequencies (Kumar and Pandey 2008) coupled with high and uniform penetration of the multicellular system (Jain 2005). The effects of gamma rays and EMS on *Chrysanthemum* were individually compared by Velmurugan *et al.* (2010). Plant material irradiated with gamma rays produced a higher percentage of chlorophyll variegation and the highest percentage of chlorophyll variegated leaves per mutated plant as compared to the EMS treated material. Gamma rays from radio nuclides such as Cobalt 60 (^{60}Co)

or Cs 137 are commonly used in food irradiation and are said to cause changes in cell wall structure and function of plant cells (Kovács and Keresztes 2002).

Gamma radiation can interact with atoms and molecules creating free radicals in the cell and these radicals could modify important components of plant cells. The morphology, anatomy, biochemistry and physiology of plant cells have been found to be the most affected, depending on irradiation dosages (Moghaddam *et al.* 2011). There are two types of irradiation available to the breeder namely; acute and chronic irradiation. Acute irradiation involves exposure at higher dosages over very short periods of time (seconds and minutes) while chronic irradiation involves exposure at relatively low dosages over extended periods (weeks or months) of time (Mba *et al.* 2010). Breeders have reported mutation success that includes higher mutation frequencies and obtaining useful mutants using different mutagens and mechanisms of mutagen application. Chronic irradiation was applied for the development of a sweet potato mutant "Nongdafu 14" with significantly higher total carotenoid content, low fibre content and better taste than the wild type in the gamma field at 142 Gy (Wang *et al.* 2007). Acute irradiation of *in vitro* nodal cuttings at 10 and 20 Gy, respectively, resulted in *Centella asiatica* mutants with significantly increased flavonoid contents, between 46.8 and 54.7% higher than the parent (Moghaddam *et al.* 2011).

Mishra *et al.* (2007) used recurrent mutagenesis on *in vitro* shoot cultures from two banana cultivars. The multiple shoot cultures were recurrently irradiated twice at 0, 10, 20 Gy with a time interval of 15 days between two successive irradiations. After each irradiation, cultures were sub-cultured on the shoot proliferation medium. Only radio sensitivity results were reported from this study and no useful mutants were mentioned. An interesting earlier report by Mehandjiev and co-workers (2001) combined both physical and chemical mutagens to induce mutations in garden pea *Pisum sativum*. They observed increased mutation frequencies in the combined treatment of gamma rays (40 Gy) and EMS (0.41%) than in single treatments. The high mutagenic efficiency of the combined treatment of gamma rays and EMS resulted in the new garden pea variety "Sredetz" with increased protein (29%) and vitamin C content as well as good productivity. Based on the successes and limitations reported above, a breeder has a choice of selecting one or combining different mutagen treatments and applications to induce mutations in different crops.

2.5.3 Plant material and sensitivity tests

The dosage to be applied for obtaining high mutation frequencies depends on the radiation type as well as the plant material treated (Brunner 1995). Initially mutants from vegetatively propagated plants were developed from rooted stem cuttings, detached leaves and dormant plants (Ahloowalia and Maluszynski 2001). But developments have led to the use of apical meristems, adventitious buds, regenerative callus cultures, somatic embryos and microspores as explants by treating them directly with a mutagen and regenerating plantlets in tissue culture (Ahloowalia and Maluszynski 2001; Jain and Suprassana 2011). The axillary *in vitro* nodal cuttings have been successfully used as plant material to induce mutations in other vegetatively propagated crops like cassava (Owoseni *et al.* 2006) and the traditional medicinal plant *Centella asiatica* (Moghaddam *et al.* 2011) through acute gamma irradiation.

Different plant materials react differently to irradiation and mutagen dosages must be optimised so as to achieve high mutation rate while avoiding serious effects on germination and plant development (Parry *et al.* 2009). The mutagen dose that achieves the optimum mutation frequency with the least possible damage is regarded as the optimal dosage (Mba *et al.* 2010). Non-conclusive observations have shown that lower dosages might have stimulatory effects on plant development (Wi *et al.* 2007) and although a high dosage may cause high mutation frequencies, it is usually accompanied by a large number of undesirable mutations in several segments of the genome (Owoseni *et al.* 2006). Harding and Mohammad (2009) confirmed this in their findings that by increasing gamma irradiation dosages (from 0 to 1200 Gy) plant development was severely affected as indicated by a significant reduction in seedling height at higher dosages on the two roselle (*Hibiscus sabdariffa* L.) varieties used. *In vitro* shoot cultures are normally irradiated at lower dosages of 0 – 20 Gy (Mishra *et al.* 2007), while *in vitro* nodal cuttings are less sensitive than the shoot cultures and can be irradiated at dosages less than 100 Gy depending on the variety sensitivity level (Owoseni *et al.* 2006; Moghaddam *et al.* 2011) and seeds are more tolerant to higher dosages and could be irradiated at dosages above 200 Gy (Spreeth and de Ronde 2004; Harding and Mohammad 2009; Taher *et al.* 2011).

To induce mutations and recover useful mutant plants, radio sensitivity tests of different cultivars and plant material must be conducted (Mishra *et al.* 2007). Dosage tests or radio sensitivity tests must be conducted so as to determine the lethal dosage which causes 30 or 50% reduction in plant height (LD_{30} / LD_{50}) when compared to the non-irradiated control for each experimental plant or variety (Brunner 1995; Owoseni *et al.*

2006; Babaei *et al.* 2010; Jain and Suprasanna 2011). This is done by exposing the specific plant material from each variety to different dosages of mutagen treatment and calculating the damage in comparison to the non-treated control. The lethality rate depends on the breeder, as some breeders use LD_{30} while others use LD_{50} (Owoseni *et al.* 2006; Mishra *et al.* 2007; Harding and Mohammad 2009; Taher *et al.* 2011; Mejri *et al.* 2012).

Previous studies on radio sensitivity tests have shown that plant/seedling height seems to be the standard measure to determine the effect of gamma irradiation on the growth and development of vegetatively propagated crops (Owoseni *et al.* 2006; Harding and Mohammad 2009; Mba *et al.* 2009). Different genotypes of the same crop react differently to different dosages, indicating genotypic effects in both seed and vegetatively propagated crops (Owoseni *et al.* 2006; Babaei *et al.* 2010; Taher *et al.* 2011; Mejri *et al.* 2012). This is supported by findings from different studies. Radio sensitivity tests conducted on different cassava genotypes using auxiliary buds of nodal cuttings from *in vitro* plantlets, showed different optimal dosages for the genotypes ranging from 12 Gy to 25 Gy respectively (Owoseni, *et al.* 2006). Again*, in vitro* nodal cuttings were used to determine lethal dosages for the two accessions of the traditional medicinal plant *Centella asiatica*. After irradiating the plant material with gamma ray dosages from 0 to 120 Gy at 10 Gy intervals, the accession CA03 was less sensitive to gamma irradiation compared to CA23 (Moghaddam *et al.* 2011). Taher *and* co-workers (2011) found different sensitivity levels to gamma rays during a study conducted to determine optimum dosages on Iranian rice genotypes. Seeds of three pure lines were exposed to different dosages of gamma rays (150, 250, 350, 350 and 450 Gy) and LD_{50} values were close to 170, 310 and 350 Gy respectively.

2.5.4 Applications of induced mutations in crop improvement initiatives

To date more than 3 000 mutant varieties have been developed globally and about 20% of the released mutants are rice mutants with only 64 of the listed mutant varieties developed in Africa (IAEA 2013). A large number of the developed mutants have been released as cultivars while others are used as parents in the development of new elite cultivars (Ahloowalia and Maluszynski 2001; Ahloowalia *et al.* 2004). China and India are the major producers of mutant varieties in the world (Jain and Suprasanna 2011). Although a large number of mutant varieties have been released worldwide, new mutant varieties released from vegetatively propagated crops are still very limited (Ahloowalia *et al.* 2004). Of all the mutant varieties listed on the database, only five mutant varieties

are listed for sweet potato and these were developed between 1986 and 1999 (IAEA 2013).

Effect of induced mutagenesis on agronomic traits and economic value

Mutations could be beneficial in agricultural systems and result in higher economic value (Majeed *et al.* 2010). Characters that could be enhanced in mutagenesis include yield; plant height; maturity; seed shattering; disease resistance; quality traits like malting quality, size and quality of starch granules and modified oil content (Ahloowalia and Maluszynski 2001; Ahloowalia *et al.* 2004). The economic value of new mutant varieties can be assessed by several parameters which include increased yield; enhanced quality; drought tolerance; increased nutritional value and improved quality (Ahloowalia *et al.* 2004). In developing countries like China, Pakistan and India, induced mutations have contributed billions of dollars to the economy (Ahloowalia *et al.* 2004).

In SA, Spreeth and de Ronde (2004) successfully developed and identified a drought tolerant mutant line from cowpea with good yield, local adaptability and other important agronomic traits through induced mutagenesis using gamma irradiation. Sen and Alikamanoglu (2012) also identified 39 drought tolerant sugar beet mutants from *in vitro* mutagenesis of shoot tips with gamma irradiation and sub-culturing of irradiated plant material to three generations before screening. The mutant lines could be used as parents to develop improved drought tolerant varieties or be released directly as mutant varieties. In Ghana, gamma irradiation of cassava stem cuttings led to the development of a mutant variety (Tek bankye) with high dry mass content of 40% and good poundability (Kharkwal and Shu 2009). Again three mutants have been isolated from cassava with different sizes of starch grain. These have economic potential for industrial use because small starch grain seems to be very suitable for bio-ethanol production (Jain and Suprasanna 2011).

Effect of induced mutagenesis on nutritional value of food crops

Induced mutation has also been successfully used to modify the biochemical pathways involved in the accumulation of essential minerals, synthesis of vitamin A precursors, starch, proteins and oil quality, hence playing a role in the improvement of human health and nutrition (IAEA TECDOC1493, 2006). The nutritional value of commercial food crops like maize, barley, soybean and sunflower has been enhanced through the successful introduction of several mutant genes into the crop genome (Jain and Suprasanna 2011). Plant products such as starch and oil have been successfully modified by mutations in genes for key biosynthetic enzymes (Wilde *et al.* 2012). More

than 700 of the released mutant varieties were developed for enhanced nutritional quality. The reported improved nutritional contents include; a new sweet sorghum mutant variety with 20% more total carbohydrates as compared to the parental lines developed in China (Jain and Suprasanna 2011).

Wang and co-workers (2007) successfully identified five root flesh colour mutant variants of sweet potato through chronic gamma irradiation and also managed to select a specific sweet potato mutant, named "Nongdafu 14" with significantly increased β-carotene content. The root flesh colour was changed from light yellow to orange, indicating increased β-carotene content. Recently, acute gamma irradiation was successful in developing mutant plants with improved starch quantity and sugar content respectively, in sweet potato (Shin *et al.* 2011).

A study was conducted to stimulate flavonoid production in the leaves and the whole plant of *Centella asiatica* by exposing *in vitro* nodal cuttings to acute gamma irradiation. Pure flavonoids are used by modern physicians to treat important diseases due to their ability to stimulate a number of hormones. Irradiated plantlets at 10 and 20 Gy exhibited significantly higher total flavonoid content than the control in eight weeks (Moghaddam *et al.* 2011). Adekola and Oluleye (2007) also conducted a study on the effect of gamma irradiation on the chemical composition of cowpea (*Vigna unguiculata* (L.) Walp) determining if the changes were desirable or detrimental. Cowpea seeds were exposed to optimum dosages of gamma irradiation at 245 Gy and putative mutants multiplied to the M_4 generation. Seeds were subjected to proximate analyses to determine the mean nutritive value of mutants. Improved characters such as enhanced protein and low moisture content as well as a reduction in non-desirable quality traits such as those that make the seed less digestible and anti-nutritive were observed in some new putative mutants.

2.5.5 *In vitro* techniques in mutagenesis of vegetatively propagated crops

It is important to utilise tissue culture techniques for fast propagation and regeneration of plantlets from the mutated sectors (Velmurugan *et al.* 2010). Vegetatively propagated crops have complicated physiology as, after mutagenic treatment, the mutation appears as a chimera and these mutations can be lost due to lack of regeneration either *in vivo* or *in vitro* (Mandal *et al.* 2000; Jacobsen and Schouten 2007). Homozygosity can be reached through numerous sexual cycles (self-pollination) in seed propagated crops but this is not possible in heterozygous vegetatively propagated crops like sweet potato. In *vitro* techniques are becoming more important for use in mutation breeding of

vegetatively propagated crops, because it controls chimera formation (Mishra *et al.* 2007; Mba *et al* 2009; Velmurugan *et al.* 2010). The *in vitro* explants are treated with mutagens and the resulting mutants are multiplied through several cycles of axillary buds to dissolve chimerism, then homohistant plants are obtained and finally screened for the desired traits (Owoseni *et al.* 2006). The combination of irradiation and *in* vitro culture has proven to be the most valuable method in obtaining desirable mutations and for rapid propagation (Wang *et al.* 2007; Velmurugan *et al.* 2010). It can speed up the breeding programme through generation of variability up to selection and multiplication of the new genotype (El-Sayed *et al.* 2007). Other advantages of *in vitro* mutation breeding are high shoot multiplication ratio resulting in efficient chimera separation and reduction in time and space (Mishra *et al.* 2007) especially when handling very large populations required in mutation breeding.

2.5.6 Protocols used for mutagenesis in sweet potato

Sweet potato breeders tend to use different plant materials and mutagen treatments to create mutant populations and develop new mutants with desirable traits. Otani *et al.* (2006) used heavy ion beam irradiation to induce mutations in sweet potato. Stem nodes with lateral buds obtained from *in vitro* plantlets were placed on LS medium and irradiated with heavy ion beams within a range of 0 to 50 Gy with either Ne-ion or C-ion beams. After irradiation, stem nodes were transferred into fresh medium and cultured for two months. Regenerated plantlets were later grown in the field for four months to evaluate morphological characters. Plants irradiated at less than 10 Gy were vigorous *in vitro* while those irradiated at dosages above 20 Gy were slow and eventually stopped growing. During field evaluations, mutations were observed on stem height, storage root number as well as weight and storage root colour. Wang *et al.* (2007) induced mutations through chronic gamma irradiation by subjecting plants from one cultivar grown in a gamma field to different dosages of 0, 0.5, 1.5 and 2 Gy per day. The total accumulated dosages over the growth period were 0, 57, 142 and 227 Gy respectively. Young shoot tips were excised from the irradiated plants after 114 days in the gamma field and grown *in vitro*. Regenerated plants were first transplanted into pots and later moved to the field. At harvesting, five root flesh colour mutants were obtained from 142 Gy and one useful mutant “Nongdafu 14” with higher total carotenoid content, increased sweetness, low fibre content and better taste than the wild type was selected.

In an experiment conducted by Shin and co-workers (2011) sweet potato stems with axillary buds were exposed to acute gamma irradiation to induce mutations. The stems were cut from the sweet potato plant grown in the glasshouse and exposed to irradiation

at 30, 50 and 70 Gy respectively. After irradiation, two or three node stems with axillary buds were cut and planted in garden soil. The axillary buds that survived the gamma irradiation were regenerated through shoot apex culture and/or re-irradiated at 80, 100 and 120 Gy of gamma rays. Culture of axillary buds resulted in the direct induction of shoots and roots and plants were easily regenerated from the irradiated axillary buds. Regenerated plantlets were evaluated in the field and after four months, sweet potato root flesh variants and many abnormalities were found. The mutants were still to be evaluated for other characteristics.

The exposure of plant material to chronic gamma irradiation resulted in desirable mutations in the form of increased carotenoid content, and consequently improved nutritional value (Wang *et al.* 2007) but the method requires exposure to gamma rays over extended periods in a gamma field, which could be a problem in SA. All three studies did not show or explain if the mutants obtained were homohistont or chimeras since evaluation was done immediately after irradiation (Otani *et al.* 2006; Wang *et al.* 2007; Shin *et al.* 2011). It is important for the breeder to consider the mutation frequency, the effectiveness of the mutagen treatment, as well as the costs and available resources when selecting a specific mutagen treatment to induce mutations in a specific crop. For example, although the application of chronic irradiation could result in mutants with changed desired traits like increased nutritional value (Wang *et al* 2007) a breeder should consider the available resources. In most instances the gamma field must be approved and constructed first to be able to give the daily dosages over the growing period.

2.5.7 Protocols used for mutagenesis in related crops

Cassava is similar to sweet potato in that it is a vegetatively propagated crop grown for its edible storage roots and it is the most important staple root crop in Africa. Owoseni and co-workers (2006) induced mutations in cassava (*Manihot esculenta* Crantz.) by exposing explants to acute gamma irradiation. *In vitro* nodal cuttings were used as explants and exposed to different doses of acute gamma irradiation (5, 10, 15, 20, 25 and 30 Gy) for radio sensitivity tests. After irradiation, each explant was placed into a conical flask containing liquid growth medium and allowed to grow on a horizontal gyratory shaker at 30 rotations per minute at 26ºC under continuous light. Data was collected after 4 – 5 weeks on plant regeneration, plantlet height, weight and number of nodes per plantlet. This report included clear presentations of radio sensitivity tests, data collection methods and it is easy to follow. Resources required in this protocol are less laborious and could save costs as compared to the chronic gamma irradiation

(Wang *et al.* 2007). The method of Owoseni *et al.* (2006) was again reported and recommended by Mba *et al.* (2010) in guidelines for mutagenesis in vegetatively propagated crops. A study was conducted by Moghaddam and co-workers (2011) on the vegetatively propagated *Centella asiatica* which is a traditional medicinal plant. *In vitro* nodal segments from two accessions were subjected to acute gamma irradiation to stimulate flavonoid production in the leaves and whole plant. Radio sensitivity tests were conducted (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 Gy) and LD_{50} values identified at 60 Gy for CA03 and 40 Gy for CA23. Mutants with significantly higher flavonoid contents than the control were identified in eight weeks. The combination of acute irradiation and direct regeneration using axillary buds is a simple mutagenesis method that allows fast generation of mutations. However, problems that could be encountered are lower mutation frequencies coupled with chimera formation (Shin *et al.* 2011).

2.5.8 Limitations in the use of induced mutations

Lack of reproducibility in induced mutagenesis is one of the main limitations of induced mutagenesis. Even the repetition and the adherence to published irradiation conditions might not result in the same mutation (Mba *et al.* 2010). It is important for the mutation breeder to take into consideration that not all mutations will express themselves phenotypically so that it can be selected and used in a breeding programme (Brunner, 1995). Mutation induction is a random process with generally low mutation frequencies and results are unpredictable without any specific selection pressure (Jain 2005; Ceballos *et al.* 2008). Thus, mutation breeding requires induction and screening of large populations. The larger the number of irradiated plant materials, the better the chance of finding a useful mutant. However, the handling and screening of large mutant populations, especially if not using targeted gene modifications, are costly, laborious and time consuming, but still important in creating a fair chance of detecting desirable mutations (Brunner 1995; Mba *et al.* 2009). It is therefore important to develop easy and quick screening methods to identify and select promising mutants from the large mutant populations generated.

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CHAPTER 3

RADIO SENSITIVITY TESTS ON THREE SWEET POTATO CULTIVARS TOWARDS *IN VITRO* MUTAGENESIS

ABSTRACT

Radio sensitivity tests were conducted to determine LD_{50} values to induce mutations in three local cream-fleshed sweet potato cultivars. Two different types of plant material, namely excised apical meristem tips and nodal cuttings obtained from *in vitro* plantlets were used as explants. Explants from each variety were subjected to different dosages of gamma irradiation from a ^{60}Co source. Data was collected 4 – 5 weeks after transplanting explants onto growth media and the parameters measured were plant regeneration, plantlet height, plantlet weight and the number of nodes per plantlet. Simple regression analyses were performed to determine the LD_{50} values for each variety compared to the non-irradiated control. Results obtained from apical meristem stems were not significant with no linear relationships observed. Regression analyses for the experiment on nodal cutting were significant and LD_{50} values were successfully determined, with a strong negative linear relationship observed between the dosages and the response of the cultivars for all parameters measured. The use of *in vitro* excised apical meristem tips to induce mutations in sweet potato was non-conclusive. Dosages for mutagenesis using *in vitro* nodal cuttings were identified for each variety; Mokone at 55 Gy, Monate at 38 Gy and Ndou at 64 Gy. Genotypic effects were confirmed by variation in lethal dosages obtained for each cultivar.

3.1 INTRODUCTION

Sweet potato is mostly popular among farmers with limited resources (Laurie *et al.* 2004) and produces more biomass and nutrients per hectare than other food crops (Woolfe 1992) making it a suitable crop to improve food security. The crop is rich in vitamins, minerals, dietary fibre and non-fibrous carbohydrates (Wang *et al.* 2007). The CSPI also classified sweet potato as the most nutritious of all vegetables (Ehler 2010) indicating the crop's significance in addressing nutrient deficiency. The OFSP types are high in β-carotene (pro-vitamin A carotenoids) and are being promoted to combat vitamin A malnutrition (Kapinga *et al.* 2007). To optimise the crop's adoption and consumption, improved varieties are required with increased micronutrient contents, acceptable dry mass composition, drought tolerance and improved yield (Tumwegamire *et* al. 2004; Laurie *et al.* 2009; Laurie 2010). Difficulties encountered in breeding vegetatively

propagated crops (incompatibility, poor flower induction and low seed set) have motivated breeders to use induced mutations as a tool in crop improvement initiatives (Broertjies and van Harten 1988). Induced mutation has proven to be a useful tool in creating desirable genetic variability in crops which can play a role in improving human health and nutrition (Ahloowalia and Maluszynski 2001). Important traits such as starch content, storage root yield, carotenoid content, disease resistance and soluble sugar content have been successfully improved in sweet potato through induced mutations (Kukimura 1986; Wang *et al.* 2007).

The first step in mutagenesis is the selection of efficient mutagens to obtain desirable mutations (Brunner 1995; Kharkwal and Shu 2009). The success rate of mutagenesis using gamma irradiation is so far high, with more than 60% of the released mutant varieties worldwide being from gamma rays, 22% from X-rays and the rest were induced through other treatments including chemical mutagens (Ahloowalia *et al.* 2004; Shu and Lagoda 2007). In a study conducted by Velmurugan and co-workers (2010) it was found that plant material irradiated with gamma rays produced a higher percentage of chlorophyll variegation and the highest percentage of chlorophyll variegated leaves per mutated plant compared to EMS treated material. Secondly, it is important for the breeder to determine the dosage levels of irradiation, because the dosage level influences the success of mutation induction and plant regeneration (Brunner 1995; Owoseni *et al.* 2006; Babaei *et al.* 2010). To achieve this, sensitivity tests must be conducted to determine LD_{50} values before bulk irradiation to avoid excessive loss of plant material due to damage caused by the irradiation treatment (Amenorpe 2010). The breeder must conduct sensitivity tests on the specific plant material and genotypes to avoid either too high or low dosages to induce mutations (Jain and Suprasanna 2011).

The mutagen dose that induces the optimum mutation frequency with the least possible damage is regarded as the optimal dosage (Mba *et al.* 2010). Usually, a useful irradiation dose is the one that results in 50% reduction of the measured parameters or mortality rate when compared to the non-irradiated control (Brunner 1995; Owoseni *et al.* 2006; Mba *et al.* 2010). Other important factors in induced mutation are the type of plant material due to different sensitivity levels and the genotype used. It has been observed that different genotypes of the same crop react differently to different dosages, indicating genotypic effects (Owoseni *et al.* 2006; Babaei *et al.* 2010; Taher *et al.* 2011).

Based on the reported high efficiency of gamma rays on mutagenesis (Brunner 1995; Jain 2005; Kharkwal and Shu 2009; Jain and Suprasanna 2011) gamma irradiation was

selected as the mutagen treatment to induce mutations in sweet potato for increased nutritional quality and improved drought adaptation. This chapter seeks to report on the results of radio sensitivity tests conducted to determine LD_{50} values for gamma rays on *in vitro* apical meristem tips and nodal cuttings of three local sweet potato cultivars.

3.2 MATERIALS AND METHODS

Three elite cream-fleshed cultivars from ARC-VOPI sweet potato breeding programme (Laurie *et al.* 2004; Laurie and Magoro 2008) namely; Mokone, Monate and Ndou (Table 3.1) with wide adaption and acceptability were selected for this study.

Table 3.1 Important characteristics of the selected cultivars

Variety	Skin colour	Flesh colour	Root shape	Dry mass content	Taste	Marketable Roots	Marketable roots yield	Total yield
				%	Score*	Number/plot	MT/ha	MT/ha
Mokone	Cream	Cream - dark cream	Round elliptic - Long oblong - Ovate	26.7	2.9	107	40	56
Monate	Cream - cream brown pink	Cream	Round elliptic - Long elliptic	26.7	2.5	84	38	65
Ndou	Cream - slight light orange	Cream - cream pale orange	Round elliptic - Obovate - Long elliptic	27.7	2.3	100	46	64

* Taste scale: 1=Excellent, 2=Good, 3=Average, 4=Poor, 5=Very poor. Source: Laurie *et al.* (2004)

3.2.1 *In vitro* propagation

The selected cultivars were propagated and multiplied from the available *in vitro* gene bank plant material at the tissue culture laboratories of ARC-VOPI in Pretoria. *In vitro* plantlets were propagated and sub-cultured using the methods reported by Alleman *et al.* (2004). Under aseptic conditions, plantlets were de-leafed and stem cuttings with one to two nodes each were used as explants. About 20 – 25 sterilised explants were placed in a tub containing 12 ml of solidified media composed of 2 g L^{-1} gelrite supplemented with 4.4 g L^{-1} MS (Murashige and Skoog) salts and vitamins (Murashige and Skoog 1962); and 30 g L^{-1} of sucrose. The pH of the media was adjusted to 5.6 before being autoclaved at 120ºC for 20 minutes (min). The cultures were kept in the growth room for 4-5 weeks at 26℃ under 16 hour continuous light and 8 hour darkness (Fig 3.1).

Fig. 3.1 Sweet potato *in vitro* propagation procedure using nodal cuttings
Top left to Bottom right: De-leafing, cutting, placing of cuttings on growth media and regenerated plantlets in the growth room.

After bacterial contamination was experienced on plant cultures, 1 ml L^{-1} of Plant Preservative Mixture (PPM™, Plant Cell Technology, Washington DC, USA) was added to the growth medium. PPM™ is a broad spectrum preservative which kills bacteria and fungi cells. It also prevents germination of spores and can eliminate endogenous bacteria if used in higher concentration (Anonymous 2010). It has two active ingredients: 5-chloro-2methyl-3 (2H)-isothiazolone at 0.1350% and 2-methyl-3(2H)-isothiazolone at 0.0412%.

3.2.2 Radio sensitivity tests

Excision of the apical meristem tips for radio sensitivity tests

Only two cultivars, Mokone and Monate were used for this experiment because Ndou was not available due to bacterial contamination during propagation. The method of Mba and co-workers (2010) was adapted for this experiment. Under aseptic conditions, apical meristem tips of Mokone and Monate were excised from four to five week old *in vitro* plantlets using the stereo microscope in a laminar flow cabinet. The excised tips were placed in Petri dishes (10 tips per dish) containing sterile distilled water and sealed with Parafilm® before being irradiated (Owoseni *et al.* 2006; Mba *et al.* 2010).

Preparation of nodal cuttings for radio sensitivity tests

For the nodal cuttings experiment, all three cultivars (Mokone, Monate and Ndou) were used for this experiment. Under aseptic conditions, four to five week week old plantlets were de-leafed and stem cuttings with two to three nodes each (both apical and basal cuttings) were used as explants (Mba *et al.* 2010). The explants were placed in Petri

dishes (10 explants per dish) containing sterile distilled water as it was done for apical meristem tips (Owoseni *et al.* 2006; Mba *et al.* 2010).

Irradiation

The irradiation service was provided by the South African National Blood Service (SANBS) in Roodepoort, Johannesburg using a ^{60}Co source. Due to reported sensitivity of different plant material to gamma irradiation (Ahloowalia and Maluszynski 2001; Kumar and Pandey 2008), lower irradiation dosages were selected for the apical meristem tips as compared to the nodal cuttings. Petri dishes containing apical meristem tips were exposed to six different treatments with lower dosages of gamma irradiation of 0, 5, 10, 15, 20 and 25 Gy respectively, with 0 Gy being the non-irradiated control. A total of 20 meristem tips placed in two Petri dishes were irradiated per treatment. The nodal cutting explants were exposed to seven different dosage treatments of 0, 20, 40, 60, 80, 100 and 120 Gy respectively, with 0 Gy being the non-irradiated control (Owoseni *et al.* 2006). A total of 40 nodal cuttings were irradiated per treatment.

Sub-culturing of cultures for plant regeneration after irradiation

This method was the same for both experiments (apical meristem tips and nodal cuttings). Both irradiated and non-irradiated explants were directly placed in a labelled test tube containing 9 ml of MS-medium each and kept in the growth room as described in section 3.2.1.

Data collection

Apical meristem tips

Data was collected on the number of fully regenerated plantlets, number of meristem tips that did not regenerate, but were still green and the number of dead meristem tips. Data was further calculated as percentage of the control as well as the actual regeneration percentage calculated on the number of regenerated plants from the treated plantlets.

Nodal cuttings

Data was collected from the individual plantlets four to five weeks after explants were transplanted into the growth media and placed in the growth room. This was done on the fully regenerated plantlets. Radiation effects were recorded in terms of the number of regenerated plantlets (counting), the number of nodes per plantlet (counting), weight of each plantlet (weighing scale) and plant height (Owoseni *et al.* 2006; Mba *et al.* 2010).

Data handling and statistical analysis

Data collected was captured in a MS Excel spread sheet, averaged for each treatment and the differences between each treatment and control were calculated and expressed as percentages (Mba *et al.* 2010). Data was further subjected to simple regression analysis using MS Excel at $P<0.05$ and the coefficient of determination (r^2) were determined for each parameter. To establish relationships between variables and dosages, correlation coefficients (r) were tested.

3.3 RESULTS

3.3.1 *In vitro* propagation

After four to five weeks of growth, only cultivars Mokone and Monate were successfully propagated and multiplied to provide enough plant material for the first experiment of radio sensitivity tests (apical meristem tips). The third cultivar Ndou had severe bacterial contamination and had to be re-propagated on a growth media added with 1ml L^{-1} PPM™ contamination. The addition of PPM™ into the growth media for sweet potato propagation successfully controlled bacterial contamination for the cultivar Ndou and resulted in enough plant material being propagated and multiplied for radio sensitivity tests using nodal cuttings only.

3.3.2 Radio sensitivity tests

Radio sensitivity test results on apical meristem tips

The regeneration of explants into full plantlets from excised apical meristem tips took four months from transplanting (Table 3.2) as opposed to the expected four to five weeks after irradiation (Owoseni *et al.* 2006; Mba *et al.* 2010). Data was only collected on the regeneration rate because of very few plantlets obtained. The actual plantlet regeneration (calculated from the number of treated explants) indicated that 22.06% of the explants from the cultivar Mokone regenerated into full plantlets while only 2.59% from the cultivar Monate regenerated. The plantlet regeneration calculated as percentage of the control also indicated a slightly higher regeneration rate for Mokone (4.33%) compared to Monate (0.50%). Non significant differences were observed between plant regeneration at different dosages for both cultivars at 5% level of significance (Table 3.2).

Table 3.2 Results of the dosage tests conducted on apical meristem tips from two sweet potato cultivars four months after transplanting onto the growth media

Variety name	Gamma-ray Dosage	Treated explants	Plantlet regeneration[a]		Actual plantlet regeneration[b]	Green meristem Tips	Dead meristem tips
	Gy	Number	Number	%[a]	%[b]	Number	Number
Mokone	0	19	4	100	21.05	4	0
	5	20	2	50	10.00	2	0
	10	20	4	100	20.00	4	3
	15	19	5	125	26.32	5	1
	20	20	1	25	5.00	1	3
	25	20	10	250	50.00	10	3
Mean		**19.67**	**4.33**	**108.33**	**22.06**	**13.67**	**1.67**
P-value				**0.3393**	**0.3567**		
R-squared*				**0.23**	**0.21**		
Adjusted R-squared*				**0.03**	**0.02**		
Monate	0	20	1	100	5.00	17	2
	5	18	0	0	0.00	16	2
	10	20	0	0	0.00	16	4
	15	20	0	0	0.00	12	8
	20	17	0	0	0.00	4	13
	25	19	2	200	10.53	12	5
Mean		**19**	**0.50**	**50.00**	**2.59**	**12.83**	**5.67**
P-value				**0.5731**	**0.5127**		
R-squared*				**0.10**	**0.11**		
Adjusted R-squared*				**-0.12**	**-0.11**		

[a]Treatment averaged and calculated as percentage of the control. [b]Actual percentage calculated for the number of regenerated plants from the total plants treated per treatment. [*]Regressions tested at 5% level of significance.

Radio sensitivity test results on nodal cuttings

Radio sensitivity tests using stem nodal cuttings were successful and LD_{50} values were determined for each variety tested. The correlation matrix showed a strong negative linear relationship between the tested dosages and each parameter measured within a variety, with an increase in gamma-ray dosages, resulting in a decrease in the plant response at $P<0.01$ (Table 3.3). The calculated (r) value observed between gamma ray dosage and:- plant regeneration was -0.86, number of nodes per plantlet -0.87, plant weight -0.92 and plant height was -0.91.

Table 3.3 Significant correlation coefficients between gamma ray dosages and parameters measured for the radio sensitivity tests using *in vitro* nodal cuttings from the three cultivars

	Gamma dosage	Plant regeneration	Number of nodes	Plant weight
Plant regeneration	-0.86**			
Number of nodes	-0.87**	0.95**		
Plant weight	-0.92**	0.94**	0.96**	
Plant height	-0.91**	0.95**	0.97**	0.99**

******P<0.01

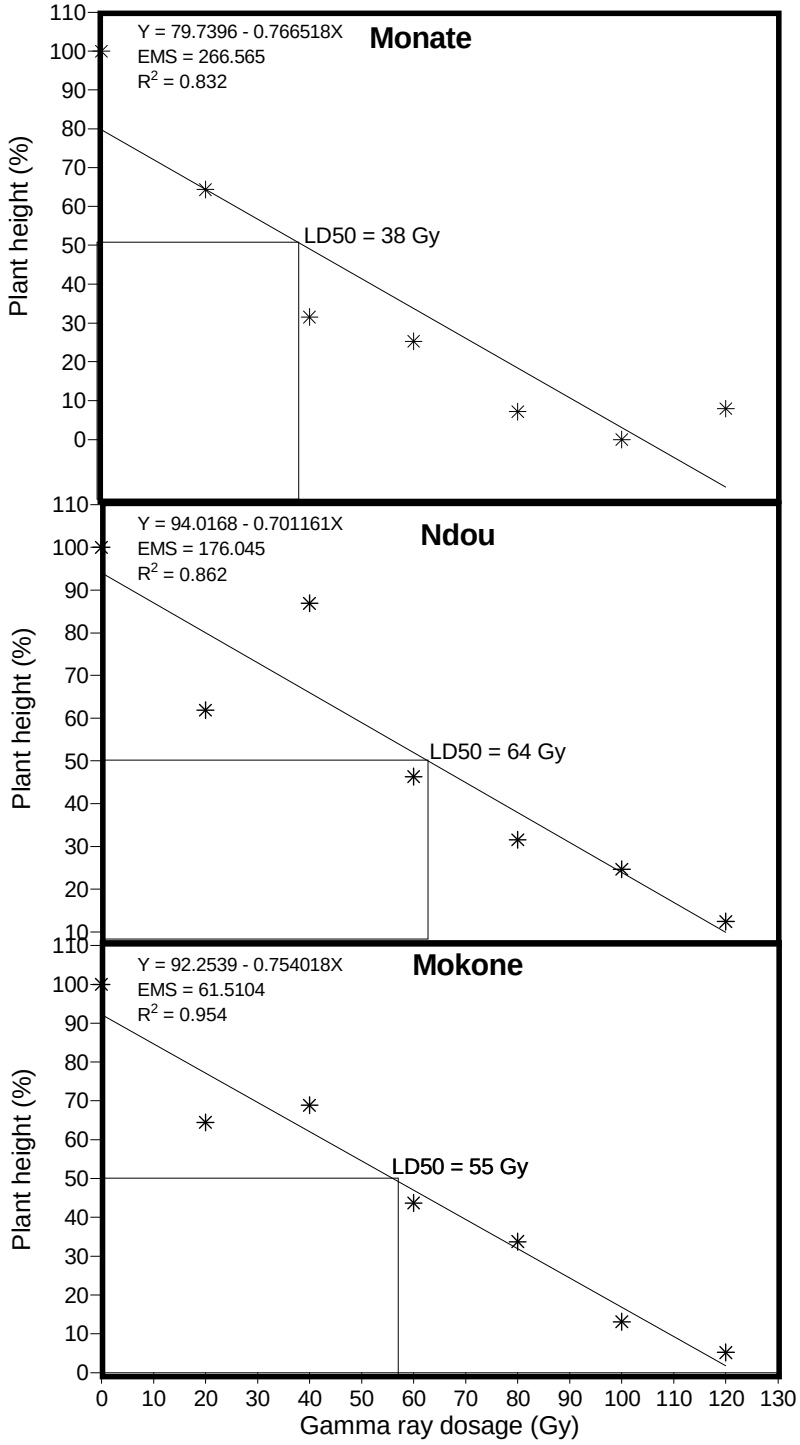
Results for radio sensitivity tests on nodal cuttings are shown in Table 3.4. Significant differences were observed for all parameters measured at P<0.01 (Table 3.4). The calculated coefficient of determination or r-squared (r^2) values obtained obtained for each parameter were higher than 0.80. The contribution of the tested gamma-ray dosages on the variation observed in plant height ranged from 83% to 95% for the three cultivars respectively.

Higher dosages of 100 Gy and 120 Gy caused significant severe reduction in all parameters. Severe reductions were observed on Monate with 100% reduction in all parameters measured at 100 Gy. Monate was more susceptible to irradiation than Mokone and Ndou (Table 3.4) and this response was the same for both nodal cuttings and excised apical meristem tips (Tables 3.2 and 3.4).

Table 3.4 Results of the dosage tests conducted on nodal cuttings of the three sweet potato cultivars at 4-5 weeks after transplanting onto the growth media

Variety name	Gamma ray Dosage	Treated explants	Plantlet Regeneration		Number of nodes per plantlet		Plant weight		Plant height	
		number	Number	%[a]	avg[b]	%[a]	avg[b]	%[a]	avg[b]	%[a]
Mokone	0	40	36	100.0	5.03	100.0	0.41	100.0	3.56	100.0
	20	40	32	88.9	3.43	68.2	0.28	68.0	2.30	64.5
	40	40	34	94.4	4.00	79.6	0.30	73.7	2.46	68.9
	60	40	26	72.2	2.60	51.7	0.19	45.0	1.56	43.7
	80	40	24	66.7	2.30	45.8	0.15	35.4	1.20	33.7
	100	40	14	38.9	0.53	10.4	0.04	9.2	0.47	13.1
	120	40	5	13.9	0.28	5.5	0.01	2.8	0.19	5.2
Mean		**40**	**24.43**	**67.85**	**2.59**	**51.6**	**0.20**	**47.73**	**1.68**	**47.01**
P-value*				**0.0013**		**0.0005**		**0.0001**		**0.0002**
R-squared*				**0.89**		**0.92**		**0.96**		**0.95**
Adjusted R-squared*				**0.87**		**0.91**		**0.95**		**0.94**
Monate	0	40	38	100.0	5.63	100.0	0.58	100.0	3.44	100.0
	20	40	22	57.9	3.75	66.7	0.35	60.0	2.22	64.3
	40	40	20	52.6	2.58	45.8	0.20	34.2	1.09	31.5
	60	40	18	47.4	1.15	20.4	0.13	22.0	0.87	25.3
	80	40	7	18.4	0.28	4.9	0.03	6.0	0.25	7.2
	100	40	0	0.0	0.00	0.0	0.00	0.0	0.00	0.0
	120	40	6	15.8	0.53	9.3	0.04	6.4	0.27	7.9
Mean		**40**	**15.86**	**41.73**	**1.99**	**26.73**	**0.19**	**32.66**	**1.16**	**33.74**
P-value*				**0.0028**		**0.0030**		**0.0040**		**0.0042**
R-squared*				**0.86**		**0.85**		**0.84**		**0.83**
Adjusted R-squared*				**0.83**		**0.82**		**0.80**		**0.78**
Ndou	0	40	33	100.0	5.1	100.0	0.37	100.0	3.35	100.0
	20	40	25	75.8	4.30	83.9	0.24	64.5	2.07	61.9
	40	40	33	100.0	5.78	112.7	0.28	73.4	2.91	86.9
	60	40	21	63.6	3.48	67.8	0.14	36.6	1.55	46.3
	80	40	16	48.5	2.54	49.5	0.13	34.9	1.06	31.5
	100	40	12	36.4	1.85	36.1	0.11	28.3	0.83	24.6
	120	40	7	21.2	0.88	17.1	0.04	9.6	0.42	12.4
Mean		**40**	**21.00**	**63.64**	**3.42**	**66.73**	**0.19**	**49.61**	**1.74**	**51.94**
P-value*				**0.0025**		**0.0048**		**0.0010**		**0.0025**
R-squared*				**0.86**		**0.82**		**0.90**		**0.86**
Adjusted R-squared*				**0.84**		**0.79**		**0.88**		**0.83**

[a]Treatment averaged and calculated as percentage of the control; [b] Calculated average for the treatment;
*Regressions tested at 95% probability



EMS – Error Mean Squares. R^2 – Coefficient of determination

Fig. 3.2 Plant height response to gamma ray dosages calculated as percentage reduction of the control for the three cultivars and their respective LD_{50} values

To determine LD_{50} values for each cultivar a graph was plotted, a line of best fit inserted (Owoseni *et al.* 2006; Mba *et al.* 2010) using data from plant height. The calculated LD_{50} values using acute gamma ray mutagenesis of *in vitro* nodal cuttings on sweet potato were 55 Gy for Mokone, 38 Gy for Monate and 64 Gy for Ndou (Fig. 3.2).

3.4 DISCUSSION

3.4.1 *In vitro* propagation

The addition of PPM™ into the growth medium was effective in controlling bacterial contamination during propagation hence the addition of 1 ml L^{-1} PPM™ to the growth media for all experiments conducted in this study. This confirms a reduced contamination observed by Jiminez and co-workers (2006) on several bamboo species after adding PPM™ to the growth medium for plantlets taken from the glasshouse.

3.4.2 Radio sensitivity tests

Radio sensitivity tests on apical meristem tips

The overall regeneration rate was very poor even with the non-irradiated control. Non-linear relationships observed, contradicted other reports on significant linear relationships observed on plant responses after irradiation at different dosages (Owoseni *et al.* 2006; Taher *et al.* 2011; Mejri *et al.* 2012) although different plant material was used.

The use of apical meristem tips in mutagenesis has been reported in general guidelines of induced mutations (Ahloowalia and Maluszynski 2001; Jain and Suprasanna 2011), but the protocols were not clear as to whether the irradiation could be done directly on the excised meristem tips or whether plantlets are irradiated and meristem tips excised later from the irradiated material as reported by other researchers (Wang *et al.* 2007). Mutagenesis was mostly done on shoot tips rather than excised apical meristem tips as reported in vegetatively propagated banana (Jancowicz-Cieslak *et al.* 2012). A report by Wang and co-workers (2007) indicated that sweet potato plants were first exposed to chronic gamma irradiation in the gamma field at different total accumulated dosages for 114 days, after which shoot tips were excised and sterilised. Shoot apices were then isolated using a dissecting microscope and cultured on the growth media. Regenerated plantlets were transplanted in pots and later evaluated in the field.

One of the limitations mentioned in induced mutagenesis is that the probability of generating desired genetic variation through induced mutations is generally low, hence

mutation breeding requires induction and screening of large populations which is costly (Brunner 1995; Jain 2005; Ceballos *et al.* 2008; Mba *et al.* 2009). For this reason, the use of excised apical meristem tips in this study would be time consuming and very costly taking into consideration the large number of populations required to create a fair chance of desirable mutations as also stated by Mba *et al.* (2009). Based on the non-significant results obtained from the two cultivars tested, Mokone and Monate (Table 3.2) it was decided not to conduct the radio sensitivity tests using apical meristem tips on Ndou. The efficient use of apical meristem tips for mutagenesis in sweet potato could therefore not be concluded in this experiment.

Radio sensitivity tests on nodal cuttings

The significantly high r^2 values observed in all parameters were in line with high r^2 values obtained by Moghaddam *et al.* (2011) and Taher *et al.* (2011). Negative linear relationships observed (Fig 3.2) indicated that an increase in gamma ray dosage resulted in a decrease in plant response for the measured parameter. This was in agreement with what Owoseni *et al.* (2006) observed on the reduction of plant weight and plant height in cassava with increased gamma ray dosages. Results reported by Taher and co-workers (2011), also showed a strong linear relationship ranging from 86 to 93% in seedling survival from radio sensitivity tests on two Iranian rice genotypes. In this experiment, lower dosages between 20 and 40 Gy did not cause much damage to the plants for the measured parameters with a slight increase observed in some parameters compared to the control (Table 3.4; Fig 3.2). This could be related to non-conclusive reports that lower dosages might have stimulatory effects on plant growth (Wi *et al.* 2007; Bolbat and Dhumal 2009) as well as an observation by Mejri *et al.* (2012) that faba bean seeds managed to repair damage caused by irradiation at dosages of less than 100 Gy.

Regeneration at higher dosages was very low, indicating that the use of higher dosages can result in severe damage on plant development as reported in other research findings (Harding and Mohammad 2009; Mejri *et al.* 2012). These results are also in agreement with what other researchers observed during radio sensitivity tests on both seed and vegetatively propagated crops. During radio sensitivity tests on two accessions of *Centella asiatica* the plant survival kept decreasing with increasing irradiation dosages for three weeks after irradiation (Moghaddam *et al.* 2011). Again the findings of Mejri and co-workers (2012) on the response of faba bean seeds after treatment with different gamma rays (0 – 700 Gy at 50 Gy interval) indicated that seeds had increasing mortality rates at dosages above 200 Gy.

The identification of LD_{50} values for the selected sweet potato cultivars using *in vitro* nodal cuttings were in line with the findings in which *in vitro* nodal cuttings have been successfully used in radio sensitivity tests for mutagenesis in cassava (Owoseni *et al.* 2006) and the traditional medicinal plant *Centella asiatica* (Moghaddam *et al.* 2011) through acute gamma irradiation.

Plant height has been reported as an effective parameter to determine LD_{50} values in mutagenesis of vegetatively propagated plants (Owoseni *et al.* 2006; Harding and Mohammad, 2009; Mba *et al.* 2009) as well as seed propagated crops (Cheema and Atta 2003). Hence, plant height was used to calculate LD_{50} values for all three cultivars and the values observed ranged between 30 and 70 Gy. Other parameters were also taken into consideration and the LD_{50} values were within the same range. The calculated LD_{50} values using acute gamma ray mutagenesis of *in vitro* nodal cuttings on sweet potato were 38 Gy for Monate, 64 Gy for Ndou and 55 Gy for Mokone respectively (Fig. 3.2).

Different LD_{50} values obtained for the different sweet potato cultivars used, confirmed results by Owoseni and co-workers (2006) who also obtained different LD_{50} values for the different cassava genotypes tested using *in vitro* nodal cuttings with values ranging from 12 to 25 Gy. Each variety responded differently to the different irradiation dosages confirming reports where different genotypes of the same crop reacted differently to different mutagen dosages due to genotypic effects on mutagenesis (Owoseni *et al.* 2006; Babaei *et al.* 2010; Moghaddam *et al.* 2011; Jain and Suprasanna 2011; Taher *et al.* 2011).

3.5 CONCLUSIONS

The integration of mutation breeding and *in vitro* techniques can broaden the spectrum of genetic variation (Mishra *et al.* 2007; Mba *et al.* 2009) and is also desirable for quick propagation of plant material over a shorter period of time. The use of excised apical meristem tips was not successful in this study, but needs to be investigated further to optimise the use and efficiency of excised apical meristem tips in sweet potato mutagenesis. This apical meristem tips method was found to be very tedious and time consuming as it requires skilled personnel for excision and could be very costly when handling large populations involved in bulk mutagenesis. The use of *in vitro* nodal cuttings enabled the identification of LD_{50} values for each of the three sweet potato cultivars investigated. The method is simple and allows easy management of large

populations within a short period of time. Due to genotypic effects observed in this study and that reported by other researchers (Owoseni *et al.* 2006; Babaei *et al.* 2010; Jain and Suprasanna 2011; Moghaddam *et al.* 2011; Taher *et al.* 2011) it is important for the breeder to conduct radio sensitivity tests on each plant material and variety of a specific crop to determine optimum dosages for each to ensure induction of efficient mutations.

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CHAPTER 4

MORPHOLOGICAL AND DROUGHT SCREENING OF GAMMA IRRADIATED SWEET POTATO MUTANT LINES TO IDENTIFY PROMISING MUTATIONS

ABSTRACT

Three elite cream-fleshed cultivars released from the South African sweet potato breeding programme were selected for mutagenesis using gamma irradiation. The selected varieties were propagated *in vitro* and subjected to bulk irradiation at the pre-determined optimal dosages to induce mutations. These explants were cultured and propagated for four generations to dissolve chimeras and obtain stable mutations. At the M_1V_4 stage, plantlets were transplanted into potting soil and hardened off in the glasshouse for morphological screening and mutation frequencies were calculated for each phenotypic change observed. Morphological changes observed on the putative mutants included: chlorophyll variegated leaves, change in leaf shape, fused veins, change in abaxial vein pigmentation, twining vines, leaf curl and changes in root flesh colour. About 410 promising mutants were identified with phenotypic and root flesh colour changes and these were selected for further evaluation in the breeding programme. A total of 110 selected Ndou putative mutant plants were multiplied and evaluated further for drought tolerance in two vegetative screening experiments. Experiment 1 entailed screening for both drought and heat tolerance while Experiment 2 evaluated mutants for drought tolerance only. In both experiments, mutant lines together with drought negative and positive control varieties were subjected to water stress in plastic boxes in a glasshouse until 70% of the plants were severely wilted or dead. Results from Experiment 1 identified 13 mutant lines with significantly improved drought and heat tolerance compared to the control cultivar Ndou. Although there were tolerant mutant lines in the second experiment, none were significantly more drought tolerant than Ndou. Mutant lines and varieties were grouped into tolerant, intermediate and sensitive for each type of stress. Sweet potato mutant germplasm with phenotypic changes, root flesh colour changes and improved drought tolerance was generated through *in vitro* mutagenesis using acute gamma irradiation.

4.1 INTRODUCTION

Sweet potato is mainly grown under rain-fed conditions by South African resource poor farmers (Laurie *et al.* 2004). Planting time and yields are highly dependent on rainfall patterns and the amount of moisture throughout the growing season. The crop is known

for its wide adaptability (Kays 2005; Aina *et al.* 2009; Lebot 2009) and inherent ability to grow under marginal conditions and still produce acceptable yields (Laurie *et al.* 2009a; Low *et al.* 2009). Although sweet potato can be grown under water stress conditions, yields under these conditions are usually lower than yields obtained under irrigated production systems. In addition, the changed rainfall distribution and prolonged water stress periods as a result of climate change (Orindi 2009) seems to threaten production of drought tolerant crops like sweet potato under rain-fed conditions (Laurie *et al.* 2009a). The impact of climate change on crop yields coupled with high population increase and reduction in cultivated land has increased food shortages especially in developing countries (Ishida *et al.* 2000; Tonukari and Omotor 2010). To reduce the impact of climatic change on food security and poverty, agricultural initiatives which promote the development and adoption of improved drought tolerant varieties are essential (Bennie and Hensley 2001; Hamdy *et al.* 2003; Tonukari and Omotor 2010).

Plant breeding could play an important role in sustainable food production, thus improving food security by developing improved drought tolerant crop varieties with improved nutritional quality. Induced mutation has been used by breeders worldwide as a tool to create genetic variation and improve important traits in both seed and vegetatively propagated crops. It has become an important crop improvement tool with no regulatory restrictions imposed like with genetically modified crops (Parry *et al.* 2009) and released mutant varieties are readily accepted by consumers (Jain and Suprasanna 2011). Moreover, induced mutations can create variability or introduce new gene combinations that were not available in the gene pool (Kumar and Pandey 2008; Majeed *et al.* 2010), thus making the technique more attractive to breeders because new traits could be added to an existing elite variety.

To induce useful mutations in the plant genome of a vegetatively propagated crop, certain principles of mutagenesis must be followed. Once optimal mutagen dosages has been determined through radio sensitivity tests (Mba *et al.* 2010), mutations can be induced by exposing or treating the specific plant material with the pre-determined dosage in bulk. Due to complicated physiology and complex genetics in vegetatively propagated crops (Brunner 1995), after treatment mutations appear as a chimera and can be lost due to lack of regeneration either *in vivo* or *in vitro* (Mandal *et al.* 2000; Jacobsen and Schouten 2007). *In vitro* techniques can be used to propagate and multiply the resulting mutants through several cycles of axillary buds to dissolve chimeras and obtain stable mutations that can be passed from one generation to the next (Owoseni *et al.* 2006; Wang *et al.* 2007; Mba *et al.* 2009; Velmurugan *et al.* 2010).

The resultant mutant population can ultimately be screened for morphological and desired traits for selection. It is important for the breeder to adopt quick screening methods which will enable identification of useful mutations easily because screening of large mutant populations, especially if not using targeted gene modifications, are costly, laborious and time consuming (Brunner 1995; Mba *et al.* 2009).

This chapter reports on the bulk irradiation of *in vitro* explants to induce mutations and create a sweet potato mutant population; propagation of irradiated explants over four generations to dissolve chimeras as well as morphological screening to identify mutants with phenotypic changes for all three varieties. Furthermore, this chapter also reports on vegetative screening for drought tolerance of mutants derived from the cultivar, Ndou. Due to a large number of mutants created and for handling purposes, only one cultivar was further evaluated for drought tolerance in this study.

4.2 MATERIALS AND METHODS

The same plant material as described in Chapter 3 section 3.2.1 was used. The mass propagation, bulk irradiation, transplanting and screening of putative mutant plants was done in sequential order with one variety at the time to allow enough space, time and resources to handle the generated large mutant populations.

4.2.1 *In vitro* propagation

Bulk irradiation was conducted on the three selected cultivars Ndou, Mokone and Monate using *in vitro* nodal cuttings as explants as described in section 3.2.2. To control bacterial contamination PPM™ was added to the growth medium at 1 ml L^{-1}. Cultures were kept in the growth room for 4-5 weeks at 26℃ under 16 hour continuous light and 8 hour darkness for regeneration of plantlets.

4.2.2 Bulk irradiation and propagation to dissolve chimeras

The adapted method of *in vitro* mutagenesis using nodal cuttings as explants (Owoseni *et al.* 2006; Mba *et al.* 2010) was used for bulk irradiation as described in Chapter 3 section 3.2.2. Explants (50) were placed in Petri dishes containing sterile distilled water and sealed with Parafilm®. A total of 1 500 nodal cuttings were irradiated for each of the three cultivars. The optimum dosages for bulk irradiation was increased slightly above lethal dosages obtained during radio sensitivity tests as discussed in Chapter 3 section 3.4.2, because it has been reported that irradiation for inducing mutations (bulk irradiation) could be over a range of ±5 of the determined lethal dosage (Owoseni *et al.*

2006) depending on plant material sensitivity. Therefore the cultivars were exposed to gamma irradiation at 80 Gy for Ndou, 60 Gy for Mokone and 40 Gy for Monate. Irradiation was done at SANBS in Roodepoort, Johannesburg using a ^{60}Co source. After irradiation, about 20 – 25 irradiated explants were placed in a tub containing 12 ml of growth media and grown under the same conditions as described in section 4.2.1. Using the same protocol for propagation, the regenerated sweet potato plantlets were propagated every 4 – 5 weeks up to four generations from M_1V_1 to M_1V_4 to dissolve possible chimeras and obtain stable mutations that could be passed from one generation to the next (Wang *et al*. 2007; Velmurugan *et al*. 2010).

Re-irradiation of Monate mutants

As a result of few morphological changes observed on Monate plantlets after irradiation in the growth room, the irradiated M_1V_4 plantlets were cut and subjected to re-irradiation (M2) using the same protocol for irradiation as described in section 4.2.2. A total of 3 000 explants from the M_1V_4 Monate mutant generation were de-leafed, cut into 2-3 nodal cuttings and re-irradiated at higher dosages. Approximately 1 500 explants were exposed to 80 Gy and 120 Gy each from the same ^{60}Co source used in section 4.2.2. After re-irradiation, all explants were transplanted into the growth media and placed under the same conditions as in section 4.2.2 and propagated every 4-5 weeks up to M_2V_4 generation. All re-irradiated explants from Monate mutants which were exposed to 120 Gy died, thus no regeneration was achieved. However, explants re-irradiated at 80 Gy regenerated with 65% loss. Approximately 1 260 of the M_1V_5 (40 Gy) and 2 100 M_2V_4 from re-irradiated (40 Gy and 80 Gy) Monate plantlets were regenerated.

4.2.3 Transplanting and morphological screening in the glasshouse

Each sub-cultured *in vitro* plantlet (M_1V_4 or M_2V_4) obtained were transplanted into a 1 L nursery bag filled with potting soil and placed in a temperature controlled glasshouse. The composition of the potting soil was a mixture of eight parts top red soil, five parts compost and 2 x 8 kg of Vermiculite. This growth medium was sterilised for 48 hours at 70ºC before use. To improve adaptability of plantlets to the new glasshouse conditions, plantlets were hardened off by covering each plantlet with a transparent plastic cup for one week (Fig. 4.1).

Fig. 4.1 Hardening off of mutant plantlets in the glasshouse

Left to Right: plants covered with transparent plastic cups; plantlets in nursery bags after removing cups and plantlets left to grow in the glasshouse before screening

After removing cups, mutant plants were maintained in the normal sweet potato maintenance programme which includes foliar fertilization with MULTIFEED P® (water soluble fertilizer for feeding and fertigation) at 8 g in 8 L of water using a watering can every two weeks plus daily irrigation with tap water. Mutant plants were allowed three months to grow and mature and then screened for signs of mutation based on phenotypic changes as compared to the control cultivars during the same growth period in the glasshouse.

Flesh colour of all putative mutants was also screened through sectioning the developing storage roots from the glasshouse mutant plants at three months after planting. The sectioned storage roots were subjected to visual assessment of the flesh colour since orange flesh colour is reported as an indication of β-carotene (Nestel *et al.* 2006; Bengtsson *et al.* 2009; Burri 2011) and this method has been employed in the ARC-VOPI breeding programme since 2003 (Laurie *et al.* 2009b). All mutants with morphological or flesh colour changes were identified, selected and grown for multiplication in seedling trays for further drought screening. Mutation frequency for each phenotypic change observed was estimated by dividing the total number of observed mutants by the total number of mutant plants in the mutant population and expressed as a percentage (Çağirgan 2006).

4.2.4 Drought screening in plastic boxes

Putative mutants derived from variety Ndou which showed apparent signs of mutation based on phenotypic and flesh colour changes were identified and multiplied in seedling trays for further drought tolerance screening in plastic boxes (155 x 77 x 23 cm). Only mutants with enough plant material, as required for the experiment, were evaluated. A quick drought tolerance screening method adapted from Singh *et al.* (1999) was used to evaluate putative mutants for drought tolerance. This method involves planting in plastic boxes and screening for drought tolerance at early vegetative stages based on survival

rate after inducing drought stress as well as days to severe wilting or death (Laurie *et al.* 2009a; Laurie *et al.* 2009b; Laurie *et al.* 2013).

Two drought screening experiments were conducted. The first experiment was planted in December 2011 evaluating both heat and drought tolerance on 77 selected putative mutants derived from Ndou. The experiment was conducted in a complete randomised design (CRD) with three replicates and six boxes per replicate. The second experiment evaluating drought tolerance only was planted in April 2012 during which 33 putative mutants derived from Ndou were evaluated. This experiment was also conducted in plastic boxes, in a CRD with four replicates and 2.5 boxes per replicate.

In both experiments non-irradiated control (Ndou); plus a negative (drought sensitive) control which consisted of Resisto, Bosbok or Letlhabula, and a positive control (drought tolerant) which was Zapallo, W-119 or Phala, were planted in each box. Apical four-node vine cuttings were used to establish the plants, each mutant line had two plants per replicate and each box had 20 plants. Cuttings were watered for 14 days to ensure plant establishment and then watering was stopped to induce stress. Wilting and dying off of plants were monitored daily and recorded until 70% of the plants have reached severe wilting or dead (Laurie *et al.* 2009a; Laurie *et al.* 2009b; Laurie *et al.* 2013).

4.2.5 Data collection and statistical analysis

During the water stress period in both experiments, observations were made on number of days to severe stem wilting or severe wilting, and number of days to severe wilting/dying for each plant in each box. At the end of the stress period data was collected on plant vigour and the number of severely wilted/dead plants was counted and expressed as percentage for each mutant line/variety. Plant vigour rating scale was 1 = dead plant, 2 = both stem and leaves severely wilted, 3 = both stem and leaves wilted averagely, 4 = only leaves wilted, stem rigid and 5 = plant still upright indicating its survival under water stress conditions. Data of number of days to death, percentage of plants with severe wilting/dead and the plant vigour rating at the end of the experiment was subjected to analysis of variance using Genstat Release 9.2 to calculate mean values and the Fisher's protected LSD test determined at 5% significance level relative to control varieties.

4.3 RESULTS

4.3.1 Bulk irradiation and morphological screening in the glasshouse

A total of 8 207 mutant plants from the three cultivars were subjected to morphological screening. Above ground morphological changes observed on Ndou included changes in leaf shape from cordate/heart to triangular and irregular outline or slight teeth, change from medium/thick vines to very thin vines, chlorophyll variegated leaves (mosaic), fused veins on the leaf, changes in vine colour from purple to green, small spiked leaves with chlorophyll variegation and leaf curl (Fig. 4.2).

Fig. 4.2 Morphological changes observed on mutant plants during screening
Left to Right: A non-irradiated plant with cordate-/heart-shaped leaves; different leaf changes observed during screening - change in leaf shape and light colour; small, curled leaves with variegated chlorophyll; and small spiked leaves with chlorophyll variegation.

Above ground changes observed on Monate were leaf changes; change from medium/thick vines to very thin vines and purple abaxial vein pigmentation. Changes observed on Mokone were leaf change from cordate to irregular outline or slight teeth and vine colour change from dark purple to green. Mutation frequencies for each phenotypic change observed are displayed in Table 4.1. Monate had the highest mutation frequency of 26.59% from the two generations [M_1V_5 (48.57%) and M_2V_4 (25.45%)] followed by Ndou with 3.60% from M_1V_4 plants and lastly Mokone with 2.23%) from M_1V_4 plants. Abaxial vein pigmentation changes were only observed on Monate derived mutants and a vine colour change from dark purple to green was also observed on one mutant derived from Mokone (Table 4.1).

Root flesh colour changes observed during sectioning of roots ranged from dark yellow, cream with light orange spots and light orange to yellowish cream with dark orange spots covering most of the flesh. A combined overall root flesh colour mutation frequency of 1.64% was obtained, with Monate at 4.24%, followed by Mokone at 1.66% and lastly Ndou at 1.18% (Table 4.1).

From 8 207 mutant plants screened, 144 Ndou, 78 Mokone and 188 putative mutants from the two generations of Monate (M_1V_5 and M_2V_4) were identified with morphological and slight flesh colour changes. An overall mutation frequency of 4.99% was obtained from screening all mutant plants derived from all three populations Table 4.1).

Table 4.1 Morphological and flesh colour changes observed on mutant plants and the mutation frequency for each mutant population

	Ndou M_1V_4		Mokone M_1V_4		Monate M_1V_5		Monate M_2V_4		Monate all stages		TOTAL	
Description	Number	%*	Number	%*	Number	%*	Number	%*	Number	%*	Number	%*
Leaf shape	20	0.50	17	0.49	9	25.7	39	5.80	48	6.79	85	1.04
Chlorophyll variegation	20	0.50	2	0.06	0	0.00	0	0.00	0	0.00	22	0.27
Vine colour	0	0.00	1	0.03	0	0.00	0	0.00	0	0.00	1	0.01
Root flesh colour	47	1.18	58	1.66	0	0.00	30	4.46	30	4.24	135	1.64
Curled leaves with some chlorophyll variegation	8	0.20	0	0.00	0	0.00	0	0.00	0	0.00	8	0.09
Dark green leaves	21	0.53	0	0.00	0	0.00	0	0.00	0	0.00	21	0.26
Leaf shape and chlorophyll variegation	2	0.05	0	0.00	0	0.00	0	0.00	0	0.00	2	0.02
Small spiked leaves and chlorophyll variegation	1	0.03	0	0.00	0	0.00	0	0.00	0	0.00	1	0.01
Twining vines (like *I. setosa*)	25	0.63	0	0.00	0	0.00	41	6.10	41	5.79	66	0.80
Purple leaf abaxial vein pigmentation	0	0.00	0	0.00	8	22.86	61	9.08	69	9.76	69	0.84
Mutants planted	4 000		3 500		35		672		707		8207	
TOTAL	144	3.60	78	2.23	17	48.57	171	25.45	188	26.59	410	4.99

* Mutation frequency calculated by dividing the number of observed mutants within the specific change by the total number of mutant plants in the mutant population within a column

4.3.2 Drought screening in plastic boxes

Experiment 1 – Drought and heat tolerance

ANOVA results for the drought and heat experiment are shown in Table 4.2. No significant differences were observed for the plant vigour rating at the end of the experiment (data not shown). However, significant differences were observed in the

number of days to severe wilting or death (P = 0.049) and in the percentage severely wilted or dead plants (P = 0.008) at the end of the experiment. The overall mean for the number of days to severe wilting/death was 44.99 with individual means ranging from 28.17 to 55.67 days. The overall mean for percentage wilted/dead plants was 90.8% with individual means ranging from 33.33% to 100% wilted plants at the end of the experiment. Means were grouped for each parameter and the two parameters combined to give the overall grouping for each mutant line/variety (Table 4.2).

The grouping from the number of days to death (DTD) identified 29 mutant lines and control varieties, Zapallo and Phala as tolerant with means ranging from 46.92 to 55.67. The control cultivar Ndou, 30 mutant lines, positive control W-119 and the negative control Letlhabula were identified as intermediate with means ranging from 42.33 and 46.67. Finally 18 mutant lines and the negative controls (Bosbok and Resisto) were identified as sensitive with means ranging from 28.17 to 40.83 days to severe wilting (Table 4.2).

The percentage wilt groupings classified 13 mutant lines and two positive controls (Zapallo and Phala) as tolerant with means ranging from 33.33% to 66.67%. The intermediate group included the control Ndou, four mutant lines and the positive control W-119 with means ranging from 72.23% to 85.57% and lastly the sensitive group included the negative controls Bosbok, Letlhabula, Resisto and 62 mutant lines with means ranging from 94.43% to 100% of severely wilted plants at the end of the experiment (Table 4.2). The overall grouping used both parameters (number of days to severe wilt/death and percentage severely wilted/dead plants) to group the entries. Thirteen mutant lines (M2, M3, M5, M23, M26, M31, M32, M33, M37, M45, M63, M64 and M70) were grouped with the positive controls Zapallo and Phala as tolerant to both drought and heat stress. Ndou and W-119 were intermediate while Bosbok, Letlhabula and Resisto were grouped with 51 mutant lines as sensitive to both drought and heat stress (Table 4.2).

Table 4.2 Vegetative drought and heat tolerance of 77 mutant lines in terms of number of days to severe wilting/dead and percentage of wilted plants at the end of Experiment 1

Entry	Variety/Mutant line	Mutant change	Drought – Heat tolerance						
Number	Name	Description	DTD[a]		Grouping	% Wilt/dead[b]		Grouping	Overall grouping
19	M26	Med twining vines	55.67	A	T	33.33	a	T	T
26	M32	Mosaic	55.33	Ab	T	66.67	bcde	T	T
31	M37	Leaf shape	53.33	Abc	T	66.67	bcde	T	T
68	M70	Leaf shape+mosaic	52.17	Abcde	T	50.0	ab	T	T
16	M23	Thin twining vines	52.17	Abcde	T	50.0	ab	T	T
61	M64	Thin twining vines	51.33	Abcdef	T	66.67	bcde	T	T
40	M45	Leaf shape	51.17	Abcdefg	T	66.67	bcde	T	T
12	M2	Leaf shape	51.00	abcdefgh	T	66.67	bcde	T	T
60	M63	Leaf shape	51.00	abcdefgh	T	66.67	bcde	T	T
++control	Phala		50.87	abcdefgh	T	55.2	ab	T	T
27	M33	Serious leaf shape	50.17	abcdefgh	T	66.67	bcde	T	T
45	M5	Leaf shape	50.00	abcdefgh	T	50.0	ab	T	T
25	M31	Mosaic	49.00	abcdefgh	T	33.33	a	T	T
++control	Zapallo		47.37	abcdefghij	T	61.1	bcd	T	T
23	M3	Flesh colour	46.67	abcdefghijk	I	66.67	bcde	T	T
53	M57	Mosaic	52.92	Abcd	T	96.71	de	S	I
75	M78	Leaf shape	51.00	abcdefgh	T	100.0	e	S	I
13	M20	Leaf shape	50.00	abcdefgh	T	100.0	e	S	I
30	M36	Leaf shape	50.83	abcdefgh	T	100.0	e	S	I
58	M61	Leaf shape	49.92	abcdefgh	T	96.71	de	S	I
42	M47	Leaf shape	49.83	abcdefgh	T	83.33	cde	I	I
76	M8	Leaf shape	49.83	abcdefgh	T	100.0	e	S	I
36	M41	Leaf shape	49.00	abcdefgh	T	100.0	e	S	I
29	M35	Leaf shape	48.83	abcdefgh	T	100.0	e	S	I
66	M69	Leaf curl	48.67	abcdefghi	T	100.0	e	S	I
77	M9	Leaf shape+mosaic	48.00	abcdefghi	T	100.0	e	S	I
20	M27	Leaf shape	47.67	abcdefghi	T	100.0	e	S	I
21	M28	Med, twining vines	47.67	abcdefghi	T	83.33	cde	I	I
28	M34	Dark green leaves	47.00	abcdefghij	T	100.0	e	S	I
46	M50	Leaf shape	46.92	abcdefghij	T	96.71	de	S	I
+control	W-119		45.13	abcdefghijk	I	72.23	cde	I	I
73	M76	Flesh colour	44.67	abcdefghijk	I	83.33	cde	I	I
Parent	Ndou		43.60	abcdefghijk	I	85.57	cde	I	I
14	M21	Thin twining vines	38.17	Ghijklm	S	83.33	cde	I	S
-control	Bosbok		38.00	Hijklm	S	95.83	de	S	S
-control	Letlhabula		43.37	abcdefghijk	I	95.83	de	S	S
1	M1	Leaf curl +mosaic	43.67	abcdefghijk	I	100.0	e	S	S
2	M10	Spiked leaves + mosaic	42.50	bcdefghijkl	I	100.0	e	S	S
3	M11	Slight leaf curl	34.33	Jklm	S	100.0	e	S	S
4	M12	Slight leaf curl	45.83	abcdefghijk	I	100.0	e	S	S
5	M13	Thin twining vines	40.83	cdefghijklm	S	100.0	e	S	S
6	M14	Dark green leaves	47.67	abcdefghi	T	100.0	e	S	S
7	M15	Thin twining vines	46.67	abcdefghijk	I	100.0	e	S	S
8	M16	Thin twining vines	33.67	Klm	S	100.0	e	S	S
9	M17	Thin twining vines	38.34	Fghijklm	S	100.0	e	S	S
10	M18	Thin twining vines	28.17	M	S	100.0	e	S	S
11	M19	Thin twining vines	48.83	abcdefgh	T	100.0	e	S	S
15	M22	Thin twining vines	42.33	bcdefghijkl	I	100.0	e	S	S
17	M24	Slight leaf curl	40.00	defghijklm	S	100.0	e	S	S

Table 4.2 (continued)

Entry	Variety/Mutant line	Mutant change	Drought – Heat tolerance						
Number	Name	Description	DTD[a]		Grouping	% Wilt/dead[b]		Grouping	Overall grouping
18	M25	Med twining vines	43.42	abcdefghijk	I	96.71	de	S	S
22	M29	Med twining vines	44.50	abcdefghijk	I	100.0	e	S	S
24	M30	Flesh colour	44.50	abcdefghijk	I	100.0	e	S	S
32	M38	Dark green leaves	38.67	Fghijklm	S	100.0	e	S	S
33	M39	Dark greenleaves	40.17	defghijklm	S	100.0	e	S	S
34	M4	Mosaic	45.67	abcdefghijk	I	100.0	e	S	S
35	M40	Dark green leaves	40.83	cdefghijklm	S	100.0	e	S	S
37	M42	Mosaic	42.33	bcdefghijkl	I	100.0	e	S	S
38	M43	Flesh colour	46.67	abcdefghijk	I	100.0	e	S	S
39	M44	Mosaic	44.00	abcdefghijk	I	100.0	e	S	S
41	M46	Leaf shape	46.67	abcdefghijk	I	100.0	e	S	S
43	M48	Leaf shape	46.67	abcdefghijk	I	100.0	e	S	S
44	M49	Mosaic	38.33	Fghijklm	S	100.0	e	S	S
47	M52	Leaf shape	39.92	defghijklm	S	96.71	de	S	S
48	M51	Thin twining vines	44.67	abcdefghijk	I	100.0	e	S	S
49	M53	Leaf shape	42.33	bcdefghijkl	I	100.0	e	S	S
50	M54	Thin twining vines	44.67	abcdefghijk	I	100.0	e	S	S
51	M55	Leaf shape	46.67	abcdefghijk	I	100.0	e	S	S
52	M56	Mosaic	44.24	abcdefghijk	I	100.0	e	S	S
54	M58	Mosaic	40.00	defghijklm	S	100.0	e	S	S
55	M59	Leaf shape	43.50	abcdefghijk	I	100.0	e	S	S
56	M6	Leaf shape	46.17	abcdefghijk	I	100.0	e	S	S
57	M60	Mosaic	46.00	abcdefghijk	I	100.0	e	S	S
59	M62	Leaf shape	43.50	abcdefghijk	I	100.0	e	S	S
62	M65	Flesh colour	35.67	Ijklm	S	100.0	e	S	S
63	M66	Med twining vines	46.67	abcdefghijk	I	100.0	e	S	S
64	M67	Severe leaf curl	40.33	cdefghijklm	S	100.0	e	S	S
65	M68	Mosaic	46.42	abcdefghijk	I	96.71	de	S	S
67	M7	Thin twining vines	34.50	Jklm	S	100,0	e	S	S
69	M71	Leaf shape	38.00	Hijklm	S	100.0	e	S	S
70	M73	Mosaic	43.50	abcdefghijk	I	100.0	e	S	S
71	M74	Mosaic	29.67	Lm	S	100.0	e	S	S
72	M75	Mosaic	43.42	abcdefghijk	I	96.71	de	S	S
74	M77	Mosaic	46.67	abcdefghijk	I	100.0	e	S	S
--control	Resisto		39.33	Efghijklm	S	94.93	de	S	S
	Mean		44.99			90.8			
	P-value		0.049			0.008			
	LSD (P=0.05)		13.05			37.8			
	CV%		18.00			25.9			
	SEM		4.673			13.56			

[a]DTD = Number of days to severe wilting or death. [b]Percentage severely wilted/dead plants at the end of the experiment. S=sensitive; I=Intermediate; T=tolerant. ns – non significant at 5% significance level. LSD-Least significant difference at 5% significance level

Experiment 2 – Drought tolerance

Results of the ANOVA (P≤0.05) for the number of days to severe wilting or death, percentage severely wilted or dead plants and plant vigour rating at the end of the stress period, are displayed in Table 4.3. The number of days to severe wilting/death

(P=0.625) and percentage wilted/dead plants (P=0.115) were not significant. Significant differences were observed for plant vigour rating (P<0.001) at the end of the stress period. Average plant vigour rating was 1.745 with means ranging from 0.125 to 3.125. Drought tolerant control varieties Zapallo, Phala and W-119; the control Ndou; two intermediate varieties Letlhabula and Bosbok and 14 mutant lines were grouped as tolerant to drought. The intermediate group comprised of mutant lines only while 12 mutant lines were grouped with the negative control Resisto as drought sensitive. No mutant line with significantly improved drought tolerance compared to Ndou was identified. However, 13 mutant lines had higher mean values (≥ 2.000) than that of Ndou (1.931). Mutant lines M32 and M26 were significantly more sensitive to drought compared to the control cultivar (Table 4.3).

4.4 DISCUSSION

The approach to increase the optimum dosages for bulk irradiation slightly above those obtained during radio sensitivity tests, proved successful, except in the case of Monate. During *in vitro* propagation few morphological changes were observed in Monate plantlets after irradiation, hence the decision to re-irradiate at higher dosages in order to increase the chance of getting improved mutations. The few changes observed could be related to a report that irradiation at lower dosages might result in non-significant morphological changes (Wi *et al.* 2007). After re-irradiation at 80 and 120 Gy respectively, all explants irradiated at 120 Gy died and did not regenerate. This was in line with reports that higher dosages of gamma irradiation can cause severe damage in plant growth and development (Wi *et al.* 2007; Harding and Mohammad 2009; Mejri *et al.* 2012) and also confirmed radio sensitivity test results obtained in Chapter 3 in which the regeneration rate of Monate at 120 Gy was less than 20%.

Poor adaptability and high mortality rates observed on Monate mutant plants after transplanting in the glasshouse could not be explained because plantlets from both single and re-irradiated treatments died. However, Monate was identified as the most sensitive variety of the three during radio sensitivity tests and this could be one reason for high mortality and poor adaptation of this variety.

Table 4.3 Vegetative drought tolerance of 33 mutant lines in terms of the plant vigour rating at the end of Experiment 2

Entry	Variety/Mutant line	Mutant change	Drought tolerance			
Number	Name	Description	DTD[a]	%Wilt/dead	Vigour[c]	Grouping
++control	Zapallo		62.12	0.0	3.125 a	T
-control	Letlhabula		57.12	18.8	3.000 ab	T
++control	Phala		58.81	12.5	2.688 abc	T
-control	Bosbok		48.75	43.8	2.500 abcd	T
15	M37	Leaf shape	58.50	25.0	2.5000 abcd	T
26	M61	Leaf shape	59.75	25.0	2.5000 abcd	T
27	M63	Leaf shape	55.50	25.0	2.5000 abcd	T
8	M23	Flesh colour	45.87	37.5	2.375 abcd	T
2	M5	Leaf shape	52.62	12.5	2.375 abcd	T
31	M71	Leaf shape	63.50	12.5	2.375 abcd	T
14	M36	Leaf shape	58.12	50.0	2.250 abcde	T
24	M55	Leaf shape	55.25	50.0	2.250 abcde	T
5	M20	Leaf shape	63.50	37.5	2.125 abcde	T
1	M3	Flesh colour	59.00	37.5	2.125 abcde	T
4	M9	Chlorophyll variegtaion and leaf shape	54.25	25.0	2.125 abcde	T
20	M48	Leaf shape	55.75	37.5	2.000 abcdef	T
25	M59	Leaf shape	51.75	25.0	2.000 abcdef	T
Parent	Ndou		57.01	32.5	1.931 abcdefg	T
22	M53	Leaf shape	61.25	37.5	1.875 abcdefg	T
+control	W-119		60.94	56.2	1.875 abcdefg	T
6	M21	Thin twining vines	70.00	12.5	1.750 bcdefgh	I
12	M33	Serious leaf shape	52.37	37.5	1.750 bcdefgh	I
16	M41	Leaf shape	50.37	37.5	1.750 bcdefgh	I
33	M78	Leaf shape	61.50	37.5	1.625 cdefgh	I
13	M35	Leaf shape	50.50	50.0	1.500 cdefgh	I
18	M45	Leaf shape	57.00	37.5	1.500 cdefgh	I
29	M66	Medium twining vines	53.37	50.0	1.500 cdefgh	I
19	M46	Leaf shape	54.50	62.5	1.375 cdefghi	S
21	M51	Thin twining vines	53.75	37.5	1.375 cdefghi	S
10	M28	Medium, twining vines	57.74	85.5	1.258 defghi	S
7	M22	Thin twining vines	60.62	50.0	1.250 defghi	S
3	M8	Leaf shape	53.87	25.0	1.250 defghi	S
28	M64	Thin twining vines	50.50	75.0	1.000 efghi	S
--control	Resisto		61.81	62.5	0.938 efghi	S
17	M42	Thin twining vines	57.37	62.5	0.750 fghi	S
23	M54	Thin twining vines	60.75	50.0	0.750 fghi	S
30	M70	Leaf shape + mosaic	57.62	62.5	0.750 fghi	S
32	M76	Thin twining vines	59.62	50.0	0.625 ghi	S
11	M32	Thin twining vines	45.87	75.0	0.500 hi	S
9	M26	Medium twining vines	48.75	100.0	0.125 i	S
Mean			56.72	41.5	1.745	
F value			0.625	0.115	<0.001	
LSD			ns	ns	1.350	

[a]DTD = umber of days to severe wilting ordeath. [b]Percentage severely wilted plants at the end of the experiment. [c]Plant vigour rating at the end of the stress period. S=sensitive; I=Intermediate; T=tolerant. ns – non significant at 5% significance level. LSD-Least significant difference at 5% significance level

Morphological screening of mutants through identification and selection of mutants based on phenotypic changes which were visible to the eye, was a simple and quick screening method. A similar method was also used to select useful sorghum mutants by Mehlo *et al.* (2013). Results from this study showed an overall low mutation frequency of 4.99% after screening of all three mutant populations for morphological changes (Table 4.1). Mehlo *et al.* (2013) selected only five mutants (0.33%) with altered phenotypes from 1 500 gamma irradiated sorghum seeds. These findings also confirm reports that mutations occur randomly with generally low frequencies, hence the importance of screening large populations to be able to increase the chance of obtaining useful mutants (Jain 2005; Ceballos *et al.* 2008). Results obtained from an induced mutation study on sweet potato studies earlier, also indicated that the use of acute irradiation on nodal stem cuttings and direct regeneration might result in low mutation frequencies (Shin *et al.* 2011). Furthermore Parry *et al.* (2009) indicated that: “polyploidy species have a high tolerance to mutations due to complementation of essential genes by homoeologus copies and thus populations saturated with mutations can be much smaller” and again, “recessive mutations in single homoeologues of a gene in these polyploids are less likely to show a phenotypic change”.

Monate had the highest mutation frequencies when compared to the other cultivars. Shin and co-workers (2011) also used re-irradiation by exposing axillary buds that survived the first gamma irradiation to re-irradiation to obtain useful sweet potato mutant plants from acute gamma irradiation. It is known that even if high dosages may result in high mutation frequencies as observed with Monate, they are usually accompanied by a large number of undesirable mutations in several segments of the genome (Owoseni *et al.* 2006) and this might be the reason for poor adaptation of Monate mutants after transplanting in the glasshouse.

Mutants with changed root flesh colour were identified and this was in line with earlier reports that the root flesh colour of sweet potato can be changed through chronic and acute gamma irradiation respectively (Wang *et al.* 2007; Shin *et al.* 2011). However, in this experiment there were no mutant plants with a uniform dark orange root flesh colour, meaning that the selected mutants might have lower β-carotene contents when compared to the dark orange varieties like the USA cultivar, Resisto (Laurie *et al.* 2012).

Results obtained from drought and heat tolerance screening (Experiment 1) identified 13 mutant lines with significantly improved drought and heat tolerance based on percentage severely wilted/dead plants at the end of the experiment compared to the control cultivar

Ndou (Table 4.2). The successful identification of improved drought tolerant mutant lines in sweet potato from *in vitro* mutagenesis supports findings by other researchers that drought tolerance could be improved in food crops through induced mutations in the form of gamma irradiation. Sen and Alikamanoglu (2012) identified 39 drought tolerant sugar beet mutants after *in vitro* mutagenesis of shoot tips with gamma irradiation and sub-culturing of irradiated plant material to three generations before *in vitro* drought screening with polyethylene glycol (PEG) 6000. In SA, Spreeth and de Ronde (2004) successfully developed and identified a drought tolerant cowpea mutant line with good yield, local adaptability and other important agronomic traits through induced mutagenesis of seeds using gamma irradiation followed by drought screening using boxes in the glasshouse.

Data from the drought tolerance screening (Experiment 2) identified no mutant line with significantly improved drought tolerance compared to Ndou (Table 4.3). Both experiments managed to group the drought tolerant varieties Zapallo, Phala and W-119 as tolerant to intermediate; and Resisto as a sensitive variety. Zapallo has been used in previous studies as a tolerant variety and Resisto as a drought sensitive variety and the findings from both experiments were in line with those found by Laurie *et al.* (2013).

Results from the two experiments in the present study cannot be compared because the stress conditions were different (drought stress only versus drought and heat stress combined). Drought and heat stress screening was conducted when temperatures were high in summer, while the drought screening procedure was conducted during the cooler months of the year during which only water stress was evaluated. The same was also observed by Laurie and co-workers (2013) in screening landraces of sweet potato. Some of the landraces were grouped as tolerant in one experiment and sensitive in the other related to the different physiological reactions to the two types of stress. Results from the quick vegetative drought screening in plastic boxes agreed favourably with field experiments, including root yield, in rain-out shelters where Resisto was found to be sensitive and W-119 tolerant to drought (Laurie *et al.* 2009a).

4.5 CONCLUSIONS

Mutagenesis in the form of acute gamma irradiation on *in vitro* nodal cuttings was successful in generating sweet potato mutant germplasm for the sweet potato breeding programme in SA. The use of *in vitro* techniques for mutagenesis and propagation, as well as the sequential handling of mutant populations, were beneficial as it allowed quick

propagation and multiplication of plantlets and easy handling of large mutant populations over a shorter period of time when compared to *in vivo* propagation. Although the overall mutation frequencies were low, which was in agreement with other reports (Jain 2005; Ceballos *et al.* 2008; Mehlo *et al.* 2013), promising mutants were identified with changed phenotypic appearance, changed root flesh colour from cream to light orange or cream with orange spots, as well as mutants with improved drought tolerance respectively. A total of 410 sweet potato putative mutants plants were identified and selected from the three mutant populations generated. These included 13 mutant lines with significantly improved drought and heat tolerance compared to Ndou.

Re-irradiation could increase mutation frequencies when using acute irradiation, but a breeder must be careful not to create undesirable mutations in several segments of the genome (Owoseni *et al.* 2006). It is important to adopt other genetic screening techniques in mutagenesis to be able to detect induced point mutations as well as to identify traits such as yield, quality, stress tolerance and disease resistance more effectively from the mutant populations (Parry *et al.* 2009) and to avoid disregarding useful mutations that could not be expressed phenotypically. The generated mutant germplasm will be included in the sweet potato breeding programme for further evaluation or used as parents in crosses and possibly released as varieties that will produce good yields under drought conditions and contribute in reducing nutrient deficiency.

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CHAPTER 5

PRELIMINARY FIELD EVALUATION AND MINERAL ANALYSIS OF SWEET POTATO MUTANT LINES

ABSTRACT

Gamma irradiation was used to induce mutations in sweet potato to improve agronomic traits and enhance micronutrient contents of a South African cream fleshed cultivar, Ndou. Selected mutant lines were evaluated for important agronomic traits in the field. Due to limited plant material, two field experiments were conducted sequentially in two localities during different seasons. Plants were left to grow under the normal sweet potato maintenance programme with scheduled irrigation, weeding and fertilization for ±5 months before harvesting. The control cultivar and 35 mutant lines were evaluated in the initial evaluation trial as single plants in a non-balanced completely randomised design at Lwamondo research station in Thohoyandou, Venda during the winter of 2012 season. At harvesting, data was collected on growth vigour and root yields, fresh storage roots were sampled and prepared for mineral and starch analyses. Samples were then peeled, freeze dried and ground into powder for the analyses performed in the laboratory at University of the Free State (UFS). The second field experiment was a preliminary yield evaluation trial planted with 36 entries, replicated three times in a randomised complete block design (RCBD) at Towoomba research station, Bela-Bela during the summer of 2013. Data was collected on phenotypic data, root yield (total, marketable and unmarketable) and root samples were collected to determine root dry mass content. Data collected from both experiments were subjected to ANOVA using GenStat® and Agrobase Gen II® respectively at the significance level of 5%. There were no significant improvements in mutant lines for Fe and Zn contents compared to the control cultivar. Thirteen mutant lines with improved Mn contents and seven mutant lines with significantly improved Mg contents were identified respectively. The mutant line M98 had the highest, significantly improved total starch content of 65.76% compared to Ndou, which had the lowest total starch content (47.42%). Based on phenotypic screening in the preliminary yield trial, mutant lines M224 and M6 had a visible changed root flesh colour from cream (control) to pale orange-yellow. Significant variation was observed in total yields and two mutant lines M96 (33.01 t ha^{-1}) and M95 (30.02 t ha^{-1}) had significantly improved total yields compared to Ndou (22.96 t ha^{-1}). Dry mass contents were significantly improved from 27.00% in the control cultivar to an average of 30.00% in the mutant lines; M47 (30.33%) and M28 (29.38%) at $P<0.05$. Identified mutant lines with improved traits will be further evaluated in advanced trials for both agronomic and nutrient contents to confirm these results and make recommendations.

5.1 INTRODUCTION

Food insecurity and malnutrition are of critical importance in developing countries and cannot be treated separately. An alarming increase of human population and unstable economic status have contributed to increased food shortages in developing countries (Ishida *et al.* 2000; Tonukari and Omotor 2010; Saltzman *et al.* 2013). Food shortages can lead to under-nutrition causing significant negative consequences and possible mortality in infants (Quinn and Bencko 2013). Micronutrient deficiency has also been a major health concern in these countries, being directly responsible for conditions such as xeropthalmia associated with VAD and anaemia caused by Fe deficiency (Hillocks 2011).

Sweet potato is one of the traditional crops that requires relatively low inputs (Jain 2005; Laurie *et al.* 2009), with the ability to grow on soils with low fertility and still produce acceptable yields (Aina *et al.* 2009; Lebot 2009), thus contributing to sustainable agricultural production. Furthermore, sweet potato is a highly nutritious crop, especially OFSP types that has high β-carotene contents and great potential to address VAD (Woolfe 1992; Wambugu 2003; Nestel *et al.* 2006; Bengtsson *et al.* 2009; Laurie *et al.* 2009; Ofori *et al.* 2009; Ehler 2010; Wolmarans *et al.* 2010; Burri 2011; Leksrisompong *et al.* 2012). Despite the crop's current potential in both food production and nutritional value, further improvement in mineral nutrient and crop yields is still essential to combat micronutrient deficiency and increase food production in developing countries like SA.

Malnutrition is one of the key issues that the South African government undertook to address since 1994 (Labadarios *et al.* 2005). To achieve this, crop genetic improvement initiatives aimed at developing varieties with high yields and enhanced nutritional value are required. Biofortification of staple crops is considered to be a cost effective, sustainable, long term supplementary approachs that could help meet the nutritional needs of rural populations in developing countries (Hillocks 2011; Saltzman *et al.* 2013). Another advantage is that these improved varieties will always be available to the poor communities to grow and consume even when the governments have shifted their focus from nutrient deficiency (Nestel *et al.* 2006). Breeders can combine conventional and biotechnology methods to exploit genes for essential nutrients (Johns and Eyzaguirre 2007) as well as improving agronomic adaptation. Biotechnology tools which include gene modification and induced mutations have so far created new opportunities in the improvement and availability of the total amount of nutrients in food crops worldwide (Jain and Suprasanna 2011). These tools are available to optimise the quality of food so

as to ensure food security and meet the nutritional needs of rural communities in developing countries (Tonukari and Omotor 2010; Jain and Suprasanna 2011).

Induced mutation has been successfully used by breeders worldwide to improve the nutritional value of commercial food crops like maize, barley, soybean and sunflower (Jain and Suprasanna 2011) resulting in more than 3000 mutant varieties released worldwide (IAEA 2013) Important characters that could be enhanced in mutagenesis include yield; plant height; disease resistance; quality traits like malting quality, size and micronutrient contents (Ahloowalia and Maluszynski 2001; Ahloowalia *et al.* 2004). Plant products such as starch and oil have also been successfully modified by mutations in genes for key biosynthetic enzymes (Wilde *et al.* 2012).

Genetic complications which include poor flowering, low seed set and incompatibility encountered in conventional breeding of sweet potato have slowed the crop's genetic improvement progress (Broertjies and van Hartem 1988; Kanju 2000) and motivated breeders to include other breeding tools in their respective programmes. Numerous mutagenesis studies on sweet potato using different mutagen treatments to obtain useful mutations were conducted from which mutant lines with altered flesh colour, increased carotenoid content and increased storage root number were identified (Otani *et al.* 2006; Wang *et al.* 2007; Shin *et al.* 2011). However, reports on the effect of induced mutation on total root yield, dry mass content and more importantly mineral nutrient contents of sweet potato are limited.

This study was aimed at evaluating the effects of gamma ray mutagenesis on yield, dry mass content, flesh colour and nutritional contents of a local cream-fleshed sweet potato cultivar Ndou.

5.2 MATERIALS AND METHODS

Mutants derived from the cultivar Ndou, were screened and promising mutants selected as described in Chapters 3 and 4. Selected mutant lines were multiplied and evaluated in the field for agronomic performance and adaptation. These were evaluated in two different trials; first the initial evaluation trial of single plants at Lwamondo in 2012 followed by the preliminary yield evaluation trial (PYT) at Towoomba in 2013.

5.2.1 Initial evaluation trial

Trial site, design, establishment and maintenance

A total of 144 Ndou mutant lines were initially evaluated at Lwamondo, Thohoyandou, Venda situated in the Limpopo province of SA (23.03979°S; 30.37280°E). The trial was planted on the 19th March 2012 and a detailed site description and climatic data are given in Table 5.1. These mutant lines were planted as single plants in a non-balanced completely randomised design replicated three times. This type of design was selected because of limited plant material and to allow all mutants to be evaluated at an initial stage so as to confirm the type of mutation and its stability.

Table 5.1 Climatic conditions during the growing period at Lwamondo in 2012

Month	Temperature[1]			Rainfall[2]
	Temperature Min ℃	Temperature Max ℃	Average ℃	Rainfall (mm)
March	17.50	32.79	25.14	17.27
April	12.98	28.61	20.80	21.34
May	11.04	28.94	19.99	1.52
June	8.47	26.49	17.48	4.06
July	8.69	26.03	17.36	1.02
August	10.42	28.71	19.57	2.03
September	13.69	25.11	19.40	78.99
Mean	11.83	25.11	19.96	18.03
Total				126.23
Highest recorded	20.69	37.99		0.25
Lowest recorded	0.67	16.15		29.46
Planting date	19 March 2012			
Harvesting date	12 September 2012			

[1]Means of daily average, minimum and maximum temperatures per month. [2]Total rainfall received monthly. Data obtained from the South African Weather Services

Plant material was propagated in seedling trays and left to grow in a glasshouse for four weeks. Seedlings with well established root plugs were used to establish the trial. Plant spacing was 1 m between rows, 0.5 m between plants, 11 plants per row with Ndou planted as a control in each row. Soil preparation was done by tractor before planting using a disc plough and rotovator. Ridges were made with a ridge maker spaced 1 m apart, 0.3 m wide and 0.3 m high. Fertilization was done according to soil analysis results (Table 5.2), but slightly lower than the optimum crop nutrient requirements. These recommendations were made to try and evaluate the performance of mutants under limited inputs because resource poor farmers do not apply optimum inputs for

sweet potato production. Supplementary irrigation was applied by sprinkler irrigation when needed and no chemical pesticide/herbicide control was applied. Weeding was done manually using hand hoes and hands.

Table 5.2 Soil analysis results and recommendations for Lwamondo

P-Bray mg kg^{-1}	Ammonium Acetate mg kg^{-1}								
P	K	Ca	Mg	Na	R ohm	Water pH	Clay %	Comments	Fertilization Applied (N=112, P=53, K=0)
3.1	444	957	573	19.6	1340	6.16	72	Ca level is relatively low. K level looks good. Mg level is high. No Na danger P is poorly accessible. N.P.K requirement = 120 kg N, 119 kg P and K was sufficient	Pre-plant: 200 kgha^{-1} Limestone Ammonium Nitrate LAN(28), 500 kgha^{-1} Super(10.5) Topdressing: 200 kgha^{-1} LAN(28)

Data collection

Data was collected on plant mortality and plant establishment ten days after planting. Growth data was collected every four weeks on plant vigour, visible disease symptoms and plant growth. At harvesting, data was collected on total storage root yield per plant; root skin and flesh colour. Marketable roots were considered to be of good quality and with mass ranging from 100 g to 1200 g. Unmarketable classes were extra small (<100 g); extra-large (>1200 g); mechanically damaged, long curved and sprouted roots; roots with defects like cracks, severe weevil damage, rat damage and rotten roots. Total yield included both marketable and unmarketable yields.

Sample preparation for nutritional analyses

Three medium sized storage roots were sampled from each treatment to determine mineral and total starch contents and prepared at ARC-VOPI. Sampled sweet potato roots were cut and quartered longitudinally, and opposite sections/quarters taken from each root. These were peeled, cut manually into small pieces and chopped in a food processor for fine texture. Chopped material was put in a zip-seal plastic bag and freeze dried. Each sample was ground and sieved through a 0.5 mm sieve.

Mineral analyses

Each powdered sample (2 g) was subjected to mineral content analysis for Zinc (Zn), Iron (Fe), Copper (Cu), Manganese (Mn), Magnesium (Mg), Calcium (Ca), Potassium (K) and Phosphorus (P). Mineral contents were determined at the UFS and mineral contents were measured through the ICP-OES determination according to Huang and Schulte (1985).

Total starch determination

Total starch determination was also done at the UFS by the polarimetric method (ISO 10520 1997). Approximately 2.5 g of the ground sample was weighed to the nearest 0.001g, and transferred to a 100 ml Erlenmeyer flask after which 50 ml 32% (v/v) Hydrochloric acid (HCl) was added. Flasks were placed in a boiling water bath for 15 min. Samples were stirred every 5 min using glass stirring rods. After 15 min, flasks were placed in a cold water bath to cool the sample to ±20℃. After cooling, each sample was transferred into 100 ml volumetric flasks using a small funnel. Fifteen ml of 4% (w/v) Tungstophosphoric acid was added and made up to 100 ml with distilled water and shaken gently. A double filtration was done with Whatman no 4 filter paper until there was more than 60 ml filtrate in a 150 ml beaker. Each sample filtrate was read with a polarimeter and this value was used to calculate the total starch percentage. The calculation was as follows:

$$\text{Starch \%} = \frac{10\,000 \times P}{L \times [a]_D^{20^\circ} \times S}$$

P = measured angle of optical rotation in degrees

L = length (dm) of the sample tube

$[a]_D^{20^\circ}$ = specific rotation of pure starch (+184.0° used for sweet potato)

S = exact mass of the sample weighed out

Data analysis

Since the trial was established with single plants in a non-balanced completely randomised design, only mutant lines with three replicates each were subjected to ANOVA using GenStat® and were included for further nutritional analyses. Means were separated using Fisher's LSD t-test at 5% significance level. Results obtained were first subjected to Pearson's correlation matrix test at 5% significance level to determine the relationship before running ANOVA using GenStat®.

5.2.2 Preliminary yield evaluation trial

Mutant lines evaluated in this trial were identified and selected based on available plant material, results of the initial screening procedures (drought and phenotypic) as described in Chapter 4 as well as their performance in Lwamondo initial evaluation trial. (Table 5.3).

Table 5.3 Selection criteria for mutants included in the 2013 Towoomba preliminary yield evaluation trial

Mutant	Initial mutation description[a]	Performance in the single plants evaluation and the drought screening experiments[b]
M18	Long thin twining vines	Average yield.
M35	Leaf shape	Intermediate drought tolerance
M33	Leaf shape	Good yield. Drought tolerant
M10	Small spiked leaves and chlorophyll variegation	Intermediate drought tolerance
M25	Long medium twining vines	Good yield. Drought experiment 1
M99	Yellow flesh	Good yield
M160	Yellow flesh	Good yield
M81	Yellow flesh, with orange spots	Flesh colour mutant but not tested before.
M26	Long medium twining vines	Drought tolerance results contradictory from the two experiments (tolerant and susceptible). Re-tested for other traits
M59	Leaf shape	Drought tolerant
M17	Long thin twining vines	Test for agronomic traits. Susceptible to drought
M2	Leaf shape	Drought tolerant in experiment 1
M16	Long thin twining vines	Test for agronomic traits. Susceptible in drought
M27	Leaf shape	Average yield. Drought tolerant
M183	Yellowish orange flesh	Flesh colour mutant but not tested before.
M6	Leaf shape	Drought tolerant
M188	Leaf shape	Good yield
M224	Leaf shape, yellowish orange flesh colour	Nice orange flesh colour
M62	Leaf shape	Drought tolerant
M96	Yellow flesh	Average yield. Re-tested for yield and flesh colour
M80	Yellow flesh	Good yield
M92	Cream, orange spots	Good yield
M47	Leaf shape	Intermediate drought tolerance
M70	Leaf shape and chlorophyll variegation	Drought tolerant
M166	Yellowish cream with slight orange spots	Flesh colour mutant but not tested before.
M100	Yellowish cream	Average yield
M90	Cream with slight orange spots	Good yield
M28	Long medium twining vines	Test for agronomic traits. Susceptible to drought
M21	Long thin twining vines	Test for agronomic traits. Intermediate drought tolerance
M45	Leaf shape	Drought tolerant
M150	Yellowish orange	Flesh colour mutant but not tested before.
M95	Light orange	Flesh colour mutant
M98	Cream with broad light orange ring	Average yield. Re-test for all agronomic traits and flesh colour
M52	Leaf shape	Test for agronomic traits. Susceptible to drought
M182	Leaf shape and twining vines	Not tested before

[a]Mutant description as determined during the morphological screening in the glasshouse at the M_1V_5 stage. [b]Mutants selected based on the overall mutant performance during the single plants evaluation trial at Lwamondo and the preliminary drought tolerance screening experiments conducted in 2011/2012

Trial site, design, establishment and maintenance

Selected mutant lines were further evaluated in the PYT at Towoomba research station (24.89907°S; 28.32332°E) situated in Bela Bela, Lim popo province (Table 5.4) during the summer of 2013.

Table 5.4 Climatic conditions during the growing period at Towoomba in 2013

Month	Temperature[1]			Rainfall[2]
	Temperature Min ℃	Temperature Max ℃	Average ℃	Rainfall (mm)
January	16.91	34.03	25.49	16.00
February	17.20	34.80	26.00	77.20
March	15.30	31.60	23.40	114.20
April	11.20	28.20	19.70	75.80
May	6.90	26.90	16.90	0.00
June	4.40	25.50	14.90	0.00
Mean	11.98	30.17	21.07	47.20
Total				283.20
Highest recorded	20.1	40.2		42.4
Lowest recorded	-0.5	20.3		0.2
Planting date	21 January 2013			
Harvesting date	21 June 2013			

[1]Means of daily average, minimum and maximum temperatures per month. [2]Total rainfall received monthly. Data obtained from South African Weather Services

Thirty six entries including selected mutant lines and the control cultivar Ndou, were evaluated in a replicated yield trial. This trial was planted on the 21st January 2013 in a RCBD replicated three times. Treatments were planted as 6 m long single rows with 20 plants each, 1.2 m spacing between rows and 0.3 m spacing between plants. Top/apical six node cuttings were used to establish the trial because they perform better compared to basal cuttings (Lebot 2009). These were planted upright in the centre of the ridge with half of the cutting in the soil and planting started at the beginning of the row. Fertilization was done according to soil analysis results (Table 5.5), but slightly lower than the optimum crop nutrient requirements. Supplementary irrigation was supplied by sprinkler irrigation when needed and no chemical pesticide/herbicide control was applied. Weeding was done manually using hand hoes and hands.

Table 5.5 Soil analysis results and recommendations for Towoomba

P-Bray mg kg^{-1}	Ammonium Acetate (mg kg^{-1})								
P	K	Ca	Mg	Na	R ohm	Water pH	Clay %	Comments	Fertilization Applied (N=144, P=47, K=182)
5.1	135	916	255	0.81	1400	6.38	24	Ca and Mg levels are relatively high. K level is relative low. N.P.K requirement = 150 kg N, 80 kg P and 245 kg K	Pre-plant: 500 $kgha^{-1}$ fertilizer mix 2:3:2 (22)), 300 $kgha^{-1}$ Potassium Chloride KCL (50) Topdressing: 400 $kgha^{-1}$ LAN(28)

Data collection

Data was collected on plant mortality, growth vigour, disease incidence and plant growth during the growing season. During harvesting, data was collected on; leaf shape, vine thickness, marketable and unmarketable yields (kg) from all 20 plants per plot, number of storage roots per plot, raw taste, root skin as well as root flesh colour. Marketable roots which were mechanically damaged during harvesting were also added to marketable yield to calculate possible marketable yield values if mechanical damage did not occur during harvesting. Sampling of two medium sized roots was done from each plot to determine dry mass content for each variety.

Determination of dry mass content

Dry mass content was determined from two medium-sized roots which were sliced and dried in the oven at 80ºC for 48 hours. Dry mass content (%) was calculated as dry weight (g) / fresh weight (g) x 100.

Data analysis

Data was first transformed for all variables in kg to t ha^{-1}. An ANOVA was conducted from data points derived from all three replicates for total yield (t ha^{-1}), average root weight (g), marketable yield (t ha^{-1}), marketable yield (%) in relation to total yield, marketable yield plus mechanically damaged yield (t ha^{-1}) and dry mass content (%) using Agrobase Gen II®. Mutant means were separated from the mean of the control cultivar using Dunnett's t-test if the F-probability of the ANOVA was found to be significant at 5% probability level.

5.3 RESULTS

5.3.1 Initial evaluation trial

Root yield

Means of root yield (g) from the initial evaluation trial in Lwamondo are shown in Table 5.6. Significant differences were observed between varieties (P=0.0155), but no mutant line had a significant higher root yield compared to the control cultivar Ndou. Means ranged from 153.33 g $plant^{-1}$ to 1420.00 g $plant^{-1}$with an overall mean of 783.22 g $plant^{-1}$. The mutant line M25 had the highest root yield of 1420.00 g $plant^{-1}$and the control Ndou gave 1067.77 g $plant^{-1}$ while M202 had the lowest root yield of 153.33 g $plant^{-1}$.

Table 5.6 Means of single plants root yield from the Lwamondo initial evaluation trial

Entry	Name	Root weight (g)	Rank	Entry	Name	Root weight (g)	Rank
5	M25	1420.00 a	1	29	M205	670.00 bcdefg	19
11	M80	1205.00 ab	2	6	M27	665.00 bcdefg	20
13	M92	1195.00 ab	3	14	M95	660.67 bcdefg	21
18	M100	1153.33 abc	4	31	M212	630.00 bcdefg	22
24	M188	1080.00 abcd	5	33	M221	620.00 bcdefg	23
22	M160	1073.33 abcd	6	10	M53	613.33 bcdefg	24
36	Ndou	1067.77 abcd	7	15	M96	610.00 bcdefg	25
35	M226	1046.67 abcd	8	19	M101	610.00 bcdefg	26
17	M99	1026.67 abcde	9	2	M13	600.00 bcdefg	27
20	M155	986.67 abcde	10	9	M47	600.00 bcdefg	28
28	M204	925.00 abcdef	11	26	M197	546.67 bcdefg	29
7	M33	855.00 abcdef	12	8	M45	492.50 cdefg	30
4	M24	840.00 abcdef	13	34	M224	480.00 defg	31
12	M90	780.00 abcdefg	14	30	M208	365.00 efg	32
25	M191	766.67 abcdefg	15	32	M214	290.00 fg	33
23	M163	733.33 bcdefg	16	21	M158	286.67 fg	34
16	M98	710.00 bcdefg	17	1	M10	275.00 fg	35
3	M18	686.67 bcdefg	18	27	M202	153.33 g	36
	Mean	**783.22**					
	P-value	**0.0155**					
	$LSD_{0.05}$	**671.08**					
	CV%	**56.71**					

Means followed by the same letter within a column are not significantly different at 5% significance level. ns – non significant at 5% significance level. LSD - Least significant difference at 5% significance level. CV – Coefficient of variation.

Mineral analysis

The correlation results between different minerals are included in Table 5.7. Results showed significantly moderate positive correlations between Mg and Mn (r=0.592) and a strong positive correlation was observed between Mg and Ca (r=0.778). A significant negative correlation was observed between Zn and Fe (r=-0.471) at 5% significance level.

Table 5.7 Pearson's correlation matrix between mineral contents

Minerals	Zn	Fe	Cu	Mn	Ca	Mg	K	P
Zn								
Fe	**-0,471***							
Cu	0,137	**0,242***						
Mn	-0,050	0,139	**0,382***					
Ca	-0,048	0,068	**0,386***	**0,497***				
Mg	0,084	-0,028	0,329	**0,592***	**0,778***			
K	0,020	0,179	-0,140	**-0,299***	**-0,265***	**-0,335***		
P	**0,435***	-0,143	**0,244***	0,163	**0,255***	**0,307***	0,136	

* P<0.05

Results for mineral contents of samples collected from the initial evaluation trial are shown in Table 5.8. Significant differences were identified between varieties for Zn (P=0.003), Mn (P=0.037), Mg (P=0.041) and P (P=0.017) while non-significant differences were observed for Fe (P=0.121), Cu (P=0.066), Ca (P=0.500) and K (P=0.664) at 5% significance level (Table 5.8).

Zinc

Results on Zn showed significant differences between contents, but no mutant line showed a significantly higher content than that of the control cultivar. The overall mean for Zn content was 0.68 mg 100 g^{-1} with means ranging from 0.54 mg 100 g^{-1} (M80) to 1.12 mg 100 g^{-1} (M98) while the content of Ndou was 1.02 mg 100 g^{-1} (Table 5.8).

Iron

Although no significant differences (P<0.05) were observed between Fe contents, mutant line M25 had the highest Fe content of 4.22 mg 100 g^{-1} while Ndou had a value of 3.76 with trial means ranging from 2.42 mg 100g^{-1} (M191) and an overall mean of 3.28 mg 100^{-1} (Table 5.8).

Table 5.8 Mineral analysis results of the initial mutant evaluation trial from Lwamondo

Entry	Name	Zn	Fe	Cu	Mn	Ca	Mg	K	P	Starch
		mg 100^{-1}	mg 100^{-1}	mg 100^{-1}	mg 100^{-1}	mg 100^{-1}	mg 100^{-1}	mg 100^{-1}	mg 100^{-1}	%
1	M10	0.68 cdefg	3.98	2.22	2.45 bcdef	196.2	75.0 defgh	1302.0	109.5 defg	57.67 cdefghi
2	M13	0.57 fg	2.90	4.23	2.33 bcdef	146.2	63.3 h	1277.0	132.2 abcdef	54.99 ghi
3	M18	0.62 efg	2.87	**6.39**	2.69 bcdef	132.9	76.7 defgh	1187.0	114.9 cdefg	**63.82 ab**
4	M24	0.78 bcdefg	3.60	0.58	2.58 bcdef	**230.7**	118.3 abc	1095.0	**161.4 a**	59.92 abcdefgh
5	M25	0.72 cdefg	**4.22**	3.93	**5.08 a**	201.2	110.0 abcde	1242.0	122.3 cdefg	54.33 hi
6	M27	0.62 efg	2.55	3.98	1.95 cdef	157.7	88.3 abcdefh	**1558.0**	121.3 cdefg	51.79 ij
7	M33	0.75 cdefg	3.50	4.63	2.57 bcdef	197.8	100.0 abcdefgh	1160.0	158.0 ab	54.56 hi
8	M45	0.75 cdefg	3.07	0.57	3.15 bcd	197.0	108.3 abcdefgh	1062.0	128.7 abcdefg	63.61 abc
9	M47	0.60 efg	4.32	4.57	2.83 bcde	209.0	96.7 abcdefgh	1412.0	123.6 cdefg	56.30 fghi
10	M53	0.69 cdefg	2.92	4.43	1.73 def	164.3	70.0 efgh	1332.0	130.8 abcdef	62.09 abcdef
11	M80	**0.54 g**	3.12	5.98	2.45 bcdef	220.7	**127.7 a**	990.0	111.7 cdefg	57.17 efghi
12	M90	0.60 efg	3.37	6.05	3.47 b	214.0	83.3 bcdefgh	1063.0	117.0 cdefg	61.08 abcdefg
13	M92	0.57 fg	2.60	2.05	2.93 bcde	228.5	96.7 abcdefgh	1285.0	130.7 abcdef	61.15 abcdef
14	M95	0.57 fg	2.73	2.33	1.83 cdef	233.0	93.3 abcdefgh	967.0	101.9 fg	61.01 abcdefg
15	M96	0.62 efg	3.35	3.60	3.28 bc	154.7	78.3 cdefgh	1098.0	107.3 efg	61.52 abcdef
16	M98	**1.12 a**	2.93	0.63	2.40 bcdef	207.3	83.3 bcdefgh	1193.0	136.7 abcde	**65.76 a**
17	M99	0.92 abc	3.35	0.68	2.97 bcde	142.2	82.7 bcdefgh	1110.0	143.2 abcd	57.38 defghi
18	M100	0.67 cdefg	3.83	4.47	2.60 bcdef	261.5	**121.7 ab**	1262.0	157.8 ab	61.08 abcdefg
19	M101	0.83 bcde	2.70	**0.50**	2.43 bcdef	177.0	90.0 abcdefgh	1072.0	110.8 cdefg	60.11 abcdefgh
20	M155	0.82 bcdef	2.88	3.47	1.57 ef	151.0	65.0 gh	1357.0	131.8 abcdef	61.73 abcdef
21	M158	0.58 efg	3.63	0.53	3.05 bcd	269.2	98.3 abcdefgh	918.0	111.7 cdefg	59.54 bcdefgh
22	M160	0.65 defg	2.88	4.00	2.05 bcdef	176.8	75.0 defgh	1242.0	126.1 bcdefg	61.15 abcdef
23	M163	0.58 efg	3.22	4.23	2.20 bcdef	**120.8**	61.7 h	1208.0	109.7 defg	63.25 abcde
24	M188	0.58 efg	3.07	3.38	2.02 bcdef	157.3	80.0 cdefgh	1235.0	134.0 abcdef	61.88 abcdef
25	M191	0.60 efg	**2.42**	2.17	2.28 bcdef	139.0	71.7 efgh	1100.0	102.5 fg	62.53 abcde
26	M197	0.77 bcdefg	4.00	2.32	2.85 bcde	207.5	91.7 abcdefgh	1012.0	144.1 abc	59.41 bcdefgh
27	M202	0.89abcd	3.85	0.55	3.12 bcd	183.0	117.7 abc	**835.0**	118.0 cdefg	59.57 bcdefgh

Table 5.8 (continued)

Entry	Name	Zn	Fe	Cu	Mn	Ca	Mg	K	P	Starch
		$mg100^{-1}$	$mg100^{-1}$	$mg100^{-1}$	$mg100^{-1}$	$mg100^{-1}$	$mg100^{-1}$	$mg100^{-1}$	$mg100^{-1}$	%
28	M204	0.57 fg	3.17	3.60	2.40 bcdef	182.3	86.7 bcdefgh	1128.0	119.5 cdefg	**64.12 ab**
29	M205	0.55 g	2.55	3.50	1.90 cdef	182.5	86.7 bcdefgh	1195.0	112.8 cdefg	**63.91 ab**
30	M208	0.67 cdefg	2.90	4.40	2.18 bcdef	114.8	68.3 fgh	1407.0	122.0 cdefg	59.12 bcdefgh
31	M212	0.57 fg	3.12	3.98	3.10 bcd	249.3	115.0 abcd	1008.0	104.5 efg	63.46 abcd
32	M214	0.62 efg	4.00	6.80	2.18 bcdef	238.8	83.3 bcdefgh	1178.0	96.4 g	59.45 bcdefgh
33	M221	0.72 cdefg	3.82	2.47	3.18 bcd	175.8	105.0 abcdefg	1080.0	141.8 abcd	62.30 abcdef
34	M224	0.65 defg	3.53	2.53	2.42 bcdef	143.5	70.0 efgh	1195.0	129.4 abcdefg	60.40 abcdefgh
35	M226	0.59 efg	3.35	5.43	2.95 bcde	186.5	112.7 abcd	1085.0	118.1 cdefg	62.49 abcde
36	Ndou	**1.02 ab**	3.76	0.56	1.28 f	127.6	68.3 fgh	1246.0	**142.0 abcd**	47.42 j
	Mean	**0.68**	**3.28**	**3.22**	**2.57**	**185.5**	**89.5**	**1169.0**	**124.6**	**59.92**
	P-value	**0.003**	**0.121 ns**	**0.066 ns**	**0.037**	**0.500 ns**	**0.041**	**0.664 ns**	**0.017**	**<0.001**
	$LSD_{0.05}$	**0.26**			**1.48**		**40.16**		**33.90**	**6.13**
	CV%	**23.2**	**22.8**	**79.9**	**35.3**	**37.9**	**27.5**	**23.5**	**16.7**	**6.3**

Means followed by the same letter within a column are not significantly different at P<0.05. ns – non significant at P<0.05. LSD - Least significant difference at P<0.05. ns – non significant. CV – Coefficient of variation. Shaded values are significantly higher than the control Ndou.

Copper

Non-significant differences were observed between Cu contents determined. An overall mean of 3.22 mg 100 g^{-1} was found and means ranged from 0.50 mg 100 g^{-1} (M100) to 6.39 mg 100 g^{-1} (M18) while Ndou had a value of 0.56 mg 100 g^{-1} (Table 5.8).

Manganese

Thirteen mutant lines had significantly improved Mn content compared to the control cultivar with means ranging from 1.28 mg 100 g^{-1} to 5.08 mg 100 g^{-1} and an overall mean of 2.57 mg 100 g^{-1}. The mutant line M25 had the highest content of 5.08 mg 100 g^{-1} while Ndou had the lowest Mn content of 1.28 mg 100 g^{-1} (Table 5.8).

Calcium

Means of Ca contents ranged from 120.8 mg 100 g^{-1} (M163) to 230.7 mg 100g^{-1} (M24) with an overall mean of 185.5 mg 100^{-1}, but the differences were not significant. The control cultivar Ndou had a mean Ca content of 127.6 mg 100 g^{-1} (Table 5.8).

Magnesium

Results showed six mutant lines (M80, M100, M24, M202, M212 and M226) with significantly higher Mg contents than Ndou (68.33 mg 100 g^{-1}) with M80 being the highest at 127.73 mg 100 g^{-1} (Table 5.8). The overall mean for Mg content was 89.50 mg 100 g^{-1} and means ranged from 61.67 mg 100 g^{-1} (M163) to 127.73 mg 100 g^{-1} (M80).

Potassium

Non-significant difference in K contents was observed, and the entries showed a mean range from 835 mg 100 g^{-1} (M202) to 1558 mg 100 g^{-1} (M27) and an overall mean of 1169 mg 100^{-1}. Ndou had a mean content of 1246 mg 100^{-1} (Table 5.8).

Phosphate

Phosphate contents were significantly different amongst varieties with an overall mean of 124.6 mg 100 g^{-1} and means ranging from 96.4 mg 100 g^{-1} (M214) to 161.4 mg 100 g^{-1} (M24). No mutant line had significantly higher P contents compared to Ndou 142.0 mg 100 g^{-1} (Table 5.8).

Total starch content

Means for total starch contents obtained are also included in Table 5.8. Total starch contents of four mutant lines M98 (65.76%), M204 (64.12%), M18 (63.82%), M205 (63.91%) were significantly higher ($P<0.001$) than that of Ndou (47.21%) with an overall mean of 59.92 and the mean range from 47.42% to 65.76%. Ndou had the lowest total starch content compared to all the evaluated mutant lines.

5.3.2 Preliminary yield evaluation trial

Morphological characteristics and root yield

Data on morphological characterisation of storage roots, raw tasting and means of yield parameters collected from the PYT are shown in Table 5.9. There was no visible variation in the general root shape of mutant lines and that of the control cultivar. There was a slight change observed in root skin colour from cream to yellow or yellow-cream in the mutants. Two mutant lines M224 and M6 had a visible root flesh colour change from cream (control) to pale orange-yellow. The taste of uncooked storage roots after harvesting was either bitter, not sweet, slight sweet intermediate sweet, edible or sweet.

Significant differences were observed for all yield parameters measured i.e. total yield ($P<0.001$), average root weight ($P<0.001$), marketable yield ($P<0.001$), marketable yield percentage ($P=0.0158$), marketable plus damaged yield ($P<0.001$) and dry mass content at $P=0.010$ (Table 5.9).

Mutant lines M96 (33.01 t ha^{-1}) and M95 (30.02 t ha^{-1}) gave significantly improved total yields compared to the control cultivar Ndou (22.96 t ha^{-1}) with means ranging from 8.93 t ha^{-1} to 33.01 t ha^{-1}. Again, M96 gave significantly improved marketable yields of 20.51 t ha^{-1} compared to 13.05 t ha^{-1} yielded by the control cultivar with means ranging from 3.10 t ha^{-1} to 20.51 t ha^{-1} (Table 5.9).

There were significant differences between all treatments in average root weight and marketable yield percentage, but no mutant line performed better than the control cultivar. Means for the average root weight obtained ranged from 69.38 g to 179.03 g while means for marketable yield percentage ranged from 26.81% to 68.78%. There were no significant differences in marketable yield percentage between mutant lines and Ndou. Mutant lines M182, M166, M99, M18, M98 and M96 had high marketable yield percentages above 60 compared to the control cultivar at 57.22% (Table 5.9).

Table 5.9 Morphological characteristics and means of yield parameters for mutant lines evaluated in the preliminary yield trial

Entry	Name	Root shape	Skin colour	Flesh colour	Raw taste[1]	Total yield[2] (tha^{-1})	Avg root weight (g)	Maketable yield (tha^{-1})	Marketable yield (%)	Mark + damaged yield (tha^{-1})
4	M96	Elliptic - Long elliptic	Cream	Yellow – Cream	Not sweet	**33.01 q**	147.80	**20.51 q**	61.86	**22.28 q**
25	M95	Round elliptic – Elliptic	Yellow	Dark cream	Bit sweet	**30.02 q**	163.14	14.93	50.2	18.68
32	M150	Elliptic - Round elliptic	Cream	Dark cream - Pale yellow	Intermediate	28.03	130.10	14.07	50.43	17.35
8	M98	Obovate – Elliptic	Yellow cream	Dark cream	Edible	26.78	146.63	16.67	61.92	**20.41 q**
11	M21	Elliptic - Obovate – Ovate	Yellow	Dark cream	Intermediate	24.39	147.31	13.91	56.99	15.85
17	Ndou q	Obovate – Elliptic	Cream	Cream	Edible	22.96	171.37	13.05	57.22	15.01
7	M166	Elliptic - Round elliptic – Obovate	Cream	Dark cream	Intermediate	22.77	113.62	14.42	62.77	15.47
18	M90	Round elliptic – Elliptic	Yellow cream	Dark cream - Pale orange	Not sweet, crunchy	22.71	154.16	11.82	51.74	15.11
29	M70	Elliptic- Round elliptic	White	White	Sweet	22.70	135.33	13.44	57.08	15.77
6	M80	Elliptic - Obovate – Ovate	Cream	Cream - Pale orange	Intermediate	22.25	156.83	13.84	59.36	15.84
1	M18	Elliptic – Obovate	Cream	Cream	Intermediate	21.95	143.64	13.58	62.22	15.27
27	M45	Elliptic - Round elliptic	Cream	Cream	Intermediate	20.86	116.91	10.25	49.59	12.87
19	M99	Long elliptic - Round elliptic-Elliptic	Cream	Dark cream – Cream	Not sweet	20.73	155.18	12.87	62.49	15.5
23	M52	Round – Oblong	Yellow – Cream	Cream	Intermediate	20.14	179.03	11.6	57.68	14.09
20	M62	Elliptic - Long irregular	Cream	Dark cream	Not sweet – Bitter	19.85	145.26	10.20	50.11	11.87
15	M100	Long elliptic -Irregular – Elliptic	Orange – Cream	Dark cream	Slight sweet	18.78	137.21	8.26	43.71	11.66
28	M35	Elliptic	Yellow	Dark cream	Slight sweet	18.58	102.36	9.86	50.01	11.89
26	M81	Elliptic - Round elliptic-Oblong	Yellow	Dark cream	Sweet	18.56	147.50	9.76	52.67	12.48
2	M182	Elliptic - Long elliptic	Yellow	Pale yellow	Intermediate	17.27	137.27	11.88	68.78	12.57
13	M188	Elliptic	Yellow	Cream - Pale yellow	Sweet	17.21	147.02	8.21	48.44	10.55

Table 5.9 (continues)

Entry	Name	Root shape	Skin colour	Flesh colour[3]	Raw taste[1]	Total yield[2] (tha^{-1})	Avg root weight (g)	Maketable yield (tha^{-1})	Marketable yield (%)	Mark + damaged yield (tha^{-1})
5	M160	Elliptic	Cream	Cream - Pale orange	Intermediate	16.72	113.85	6.54	38.85	10.05
16	M25	Elliptic – Obovate	Yellow	Cream	Not sweet	16.47	143.52	8.76	51.13	9.99
31	M16	Round elliptic - Elliptic – Ovate	Cream	Pale yellow - Dark cream	Edible	16.37	122.26	8.71	52.86	10.78
36	M59	Elliptic	Cream	Cream	Not sweet	16.29	124.25	9.18	55.86	11.10
24	M224	Elliptic - Round elliptic	Brownish orange – Cream	**Pale orange - Dark yellow**	Slight sweet	16.14	127.48	9.57	58.45	11.07
3	M27	Elliptic - Round elliptic	Yellow	Dark cream	Edible	15.42	107.50	9.19	59.43	9.72
14	M92	Elliptic - Obovate	Yellow	Dark cream	Intermediate	15.06	133.81	8.71	58.98	10.34
21	M28	Elliptic - Ovate – Obovate	Cream	Dark cream	Intermediate	13.74	104.73	6.41	46.31	7.43
12	M26	Round elliptic- Elliptic	Cream	Dark cream - Pale orange	Tasty – Salty	13.69	95.10	7.50	55.84	8.37
9	M33	Obovate	Cream	Dark cream - Pale orange	Intermediate	13.56	113.68	7.05	52.48	8.53
22	M17	Ovate – Elliptic	Cream	Pale yellow	Intermediate	11.93	111.43	7.06	59.26	7.53
30	M183	Round elliptic – Elliptic	Cream	Cream – White	Salty – Sweet	11.31	69.38	3.10	26.81	4.11
34	M47	Elliptic- Long elliptic – Ovate	Cream	Dark cream	Intermediate	11.11	106.98	4.63	41.08	5.92
10	M2	Elliptic	Cream	Dark cream	Intermediate	10.87	94.23	6.25	50.10	7.1
33	M10	Elliptic – Obovate	Yellow	**Yellow – Cream**	Intermediate	10.76	118.73	5.19	49.59	6.95
35	M6	Ovate – Elliptic	Cream	**Pale orange –Yellow**	Intermediate	8.93	132.05	4.09	46.06	5.54
	Mean					**18.55**	**130.46**	**10.14**	**53.29**	**12.09**
	P-value					**<0.001**	**<0.001**	**<0.001**	**0.0158**	**<0.001**
	$LSD_{0.05}$					**5.65**	**29.82**	**4.51**	**13.95**	**4.18**
	CV %					**22.36**	**16.79**	**32.64**	**19.23**	**25.44**

[1]Taste of uncooked storage roots. [2]Means ranked from the highest to the lowest. Means followed by the letter (q) differ by 1-sided LSD from the means of the control; LSD - Least significant difference at 5% significance level. CV – Coefficient of variation. Shaded values are significantly higher than Ndou and colour changes from cream to orange-yellow.

Dry mass content

Means for dry mass contents from the PYT samples are shown in Table 5.10. Significant differences were observed between dry mass contents of different varieties (P=0.010). Again there were significant differences between mutant lines and the cultivar Ndou. The overall mean was 27.31%. Means ranged from 23.82% to 30.33%. Two mutant lines had significantly improved dry mass contents compared to Ndou. Mutant lines M47 with 30.33% and M28 with 29.38% dry mass contents were significantly higher than Ndou at 27.00% (Table 5.10). Mutant line M70 had the lowest and significantly reduced dry mass content compared to Ndou at 23.82%.

Table 5.10 Means for dry mass contents for mutant lines evaluated in the preliminary yield trial

Entry	Name	[1]Dry mass %	Rank	Entry	Name	Dry mass %	Rank
34	M47	30.33 q	1	9	M33	27.15	19
21	M28	29.38 q	2	5	M160	27.12	20
3	M27	29.10	3	28	M35	27.07	21
16	M25	28.88	4	17	Ndou q	27.00	22
23	M52	28.86	5	35	M6	26.89	23
26	M81	28.63	6	12	M26	26.87	24
15	M100	28.55	7	25	M95	26.87	25
36	M59	28.33	8	2	M182	26.85	26
14	M92	28.29	9	4	M96	26.80	27
32	M150	28.20	10	6	M80	26.68	28
13	M188	28.02	11	19	M99	26.43	29
22	M17	27.91	12	7	M166	25.80	30
27	M45	27.85	13	30	M183	25.77	31
8	M98	27.79	14	10	M2	25.47	32
1	M18	27.79	15	24	M224	25.43	33
18	M90	27.70	16	20	M62	25.33	34
11	M21	27.46	17	33	M10	25.21	35
31	M16	27.50	18	29	M70	23.82	36
	Mean	**27.310**					
	P-value	**0.010**					
	$LSD_{0.05}$	**2.2946**					
	CV%	**6.17**					

[1]Means ranked from the highest to the lowest. Means followed by the letter (q) differ by 1-sided LSD from the means of the control; LSD - Least significant difference at 5% significance level. CV – Coefficient of variation.

5.4 DISCUSSION

Yield

Total yields obtained from the initial evaluation trial at Lwamondo were generally low (Table 5.6). Mean root yield per plant obtained from single plants was 783.22 g per plant compared to the mean obtained from the PYT of 1288.0 g per plant (Tables 5.6 and 5.9). Seedlings were used to establish this trial and it has been reported that apical cuttings results in better growth and development of plants than basal cuttings or seedlings (Lebot 2009). Another reason could be the low total amount of rainfall received during the growing season (126.23 mm) as indicated in Table 5.1. Although sweet potato is an inherently drought tolerant crop, sufficient water is required to obtain good yields and better storage root quality because prolonged stress seems to affect yields negatively (Ekanayake *et al.* 1988; van Heerden and Laurie 2008).

Supplementary irrigation was applied at both trials using a sprinkler irrigation system, but the system at Lwamondo had a number of problems during the growing season, which could be related to poor plant development and eventually lower yields. Yield recommendations could not be made from the initial evaluation trial at Lwamondo because single plants were evaluated and also because of lower yields as a result of irrigation challenges experienced.

Promising high yielding mutants were identified from the PYT planted at Towoomba in 2013. Two mutant lines M96 and M95 gave significantly improved total yields with M96 yielding 33.01 t ha^{-1} and M95 giving 30.02 t ha^{-1} compared to the control cultivar (Ndou) that yielded 22.96 t ha^{-1} (Table 5.8). Marketable yields were also significantly improved in the mutant line M96 (20.51 t ha^{-1}) compared to the control (13.05 t ha^{-1}). Improved sweet potato yields through mutagenesis are in line with a review report by Chakraborty and Paul (2013) that induced mutations has great potential to increase yield and ultimately food production.

Mineral contents

Variation in mineral contents observed in this study (Table 5.11) was different to that found by other researchers on sweet potato (Woolfe 1992; Ishida *et al.* 2000; Laurie *et al.* 2012). This could be explained by different plant materials used and the fact that different genotypes were tested. It was also interesting to note the difference in mean ranges obtained by Woolfe (1992) and Laurie *et al.* (2012) especially in Zn and P

contents even though they both used raw material. This confirms findings from a similar study by White and co-workers (2012) which revealed that Zn concentrations in potato tubers could be influenced by environmental effects as well as nitrogen fertilization applied. The mean range for Mg content obtained in this study (61.67 – 127.73 mg 100 g^{-1}) was higher than that obtained from other studies in sweet potato (Table 5.11). This could be related to the relatively high Mg contents found in the soil before planting (Table 5.2), hence it could be essential to test the soil for all nutrient elements before conducting nutrient analysis studies on root or tuber crops.

Table 5.11 Mean range for mineral contents obtained from mutant lines and from other sweet potato studies

Mineral	Laurie *et al.* (2012) raw roots* (mg 100g^{-1})	Ishida *et al. (*2000) freeze dried powdered samples (mg 100 g^{-1})	Woolfe (1992) raw roots (mg 100 g^{-1})	Mutant lines 2013 freeze dried, powdered samples (mg 100 g^{-1})
Fe	0.73 – 1.26	-	0.16 – 0.94	2.42 – 4.22
Zn	0.51 - 0.69	0.25 – 0.39	0.27 – 1.89	0.54 – 1.12
Cu		0.15 – 0.30	0.08 – 0.28	0.50 – 6.39
Ca	34 – 63	68 – 73.3	8 – 75	120.8 – 230.7
Mg	15 – 37	26.7 – 27	18 – 36	61.67 – 127.73
Mn			0.5 – 2.6	1.23 – 5.08
P	28 – 51	40 – 42.7	41 – 70	96.4 – 161.4
K	191 – 334	235 – 502	129 – 382	835 – 1558

Iron and Mn contents were non-significantly correlated (r=0.139) but in the positive direction (Table 5.9). This was similar to the positive correlation found between Fe and Mn contents of chickpea mutants generated from lower dosages of gamma and EMS treatments (Kozgar *et al*. 2012). The mean range for Fe contents (2.42 – 4.22 mg 100 g^{-1}) obtained from this study were higher than those obtained by Laurie and co-workers (2012) with means ranging from 0.73 – 1.26 mg 100 g^{-1} from raw fresh material as well as those obtained by Woolfe (1992) with means ranging from 0.16 – 0.94 mg 100 g^{-1}. This variation is not concerning because both Laurie *et al.* (2012) and Wollfe (1992) found different values using fresh raw material and the analysis on mutant lines was done on dried plant material. Mineral contents obtained from mutant lines (Table 5.8) did not vary from those observed from the control cultivar. Hence mutant selection can be made relative to the mineral contents obtained in this study only.

A moderate positive correlation (r=0.592) was observed between Mg and Mn contents (Table 5.7). This correlation was also confirmed by four mutant lines; M25, M202, M80 and M226 which had significantly higher contents for both Mg and Mn. There was a

significant variation for Mg and Mn contents in mutant lines and the control cultivar Ndou. Manganese contents were significantly improved in 13 mutant lines. The mutant line M25 had significantly improved Mn contents of 5.08 mg $100g^{-1}$ compared to Ndou (1.28 mg 100 g^{-1}). This was in agreement with an increased Mn content observed in high yielding chickpea mutants treated with lower dosages of gamma rays and EMS mutagens (Kozgar *et al*. 2012).

Morphological changes

Two mutant lines M224 and M6 had a visible root flesh colour change from cream (control) to pale orange-yellow. Root flesh colour changes were observed after exposing sweet potato stems with axillary buds to acute gamma irradiation. This confirms a report by Wang and co-workers (2007) who managed to identify five root flesh colour mutant lines in sweet potato through chronic gamma irradiation. Although M224 and M6 had a desired flesh colour change from cream to pale orange-yellow, negative effects were observed with significantly reduced total yields compared to the control cultivar (Table 5.9). The two mutant lines yielded significantly lower than the control cultivar Ndou (22.96 t ha^{-1}) with M224 yielding 16.14 t ha^{-1} and M6 giving the lowest yield of 8.93 t ha^{-1}. This confirms reports that induced mutations might also cause undesirable mutations in several segments of the genome (Harding and Mohammad 2009) and therefore it is important to evaluate mutant plants for all important traits before selection.

Dry mass content

Two mutant lines M47 and M28 had significantly improved dry mass contents compared to the control cultivar Ndou (27%). Mutant line M47 gave high dry mass contents above 30% which is above the South African breeding programme's target of >25% (Laurie *et al*. 2009). The improvement of dry mass content in storage root crops through gamma irradiation is in line with a report by Kharkwal and Shu (2009) in which gamma irradiation of cassava stem cuttings led to the development of a mutant variety (Tek bankye) with high dry mass content of 40%.

Total starch

Four mutant lines (M98, M204, M18 and M205) had significantly higher total starch content compared to the control (Table 5.8). Mutant line M98 had a higher starch content confirming earlier findings on sweet potato mutagenesis. Shin and co-workers (2011) identified mutant plants with improved starch quantity from acute gamma irradiation in sweet potato.

It was generally observed that based on the evaluated traits, promising mutant lines were identified with one or two improved characteristics, for example M47 had improved dry mass mass (Table 5.10) and Mn contents (Table 5.8). Mutant lines M25, M202, M80 and M226 had significantly higher contents for both Mn and Mg compared to the control cultivar (Table 5.10). No mutant line was observed with more than two significantly improved characteristics from the evaluated lines. This confirms reports that induced mutation is aimed at creating mutagenesis through altering one or two major traits and maintaining the major genetic composition of the crop (Ahloowalia *et al.* 2004; Owoseni *et al*. 2006; Babaei *et al*. 2010).

5.5 CONCLUSIONS

Results from this investigation has proven that mineral contents, total starch, root flesh colour, root yield and dry mass content could be improved through gamma ray mutagenesis using *in vitro* nodal cuttings. The initial evaluation of mineral contents from single plants showed significant improvement in some nutrient mineral contents. However, there were no significant improvements observed in mutant lines for contents of the two most deficient micronutrients in human diets, Zn and Fe. On the other hand, significant improvements were observed in mutant lines for both Mn and Mg respectively. Thirteen mutant lines with improved Mn contents as well as seven mutant lines with improved Mg contents respectively were identified compared to the control cultivar. M25 had the highest significantly improved Mn content (5.08 mg 100 g^{-1}) and M80 had the highest Mg contents (127.7 mg 100 g^{-1}). Total starch contents were also significantly improved in four mutant lines (M98, M204, M205 and M18) from 47.42% of Ndou to >65% in M98. An increase in total starch will increase the use of sweet potato in industrial products such as pharmaceuticals, bio-based plastics and alcohol (Moorthy *et al.* 2010).

Mutant lines M224 and M6 had a visible changed root flesh colour from cream to pale orange-yellow. Two mutant lines (M96 and M95) were identified with significantly improved total yields. The observed yield improvement could be significant in increasing the crop's potential to produce enough food and hence address food security. Moreover, the economic value of the crop could also be improved through increased marketable yields as observed in M96. Dry mass contents were significantly improved from 27.00% in the control to an average of more than 30.00% as observed in the mutant lines M47 (30.33%) and M28 (29.38%). Improved dry mass could result in longer

shelf life after harvesting and increased consumption of sweet potato since it is known that high dry mass content is associated with dry good taste (Lebot 2009) which preferred by most adult Africans (Tumwegamire *et* al. 2004).

The adoption of induced mutation techniques in sweet potato improvement initiatives could help improve important traits in sustainable food production and increased nutrient availability in staple crops. Identified mutant lines will be further evaluated in advanced trials for both agronomic and nutrient contents including the quantification of β-carotene contents in mutant lines with changed root flesh colour for recommendations and further selections.

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GERERAL CONCLUSIONS AND RECOMMENDATIONS

This study was aimed at applying mutagenesis in the form of gamma irradiation as a tool that can induce mutations that will result in improved sweet potato varieties with high yield, enhanced nutritional quality and drought adaptation. Improved varieties would be included in the South African breeding programme aimed at sustainable food production and combatting nutrient deficiency.

Three local cream-fleshed cultivars (Ndou, Monate and Mokone) were selected for this study and these were propagated *in vitro* for *in vitro* mutagenesis procedures. The first objective of the study was to determine lethal dosages at which mutations could be induced for each cultivar. The reported *in* vitro mutagenesis method on vegetatively propagated crops using nodal cuttings (Owoseni *et* al. 2006; Mba *et* al. 2009) and excised apical meristem tips as explants was adapted and applied in this study. Radio-sensitivity tests were conducted using both apical meristem tips and nodal cuttings for each cultivar.

The use of excised apical meristem tips and its efficiency in sweet potato mutagenesis was non-conclusive in this study because of low regeneration rates observed after irradiation even on non-irradiated material. The use of *in vitro* nodal cuttings on the other side enabled successful calculation of LD_{50} values for each cultivar investigated. The method seemed to be simple and allowed easy handling and management of large populations within a short period of time. The regression analysis significantly showed negative linear relationships above 85% between the tested dosages and the response of each cultivar for all parameters measured. An increase in gamma ray dosage resulted in a decrease in the plant response for all three cultivars and regeneration was very low at higher dosages of 100 and 120 Gy in line with reports that higher dosages can result in severe damage on plant development (Harding and Mohammad 2009; Mejri *et al.* 2012). The calculated LD_{50} values for each cultivar were 64 Gy for Ndou, 38 Gy for Monate and 55 Gy for Mokone. The variation observed in LD_{50} values confirmed reports on the effect of genotypes on mutagen treatment (Owoseni *et al.* 2006; Babaei *et al.* 2010; Moghaddam *et al.* 2011; Jain and Suprasanna 2011; Taher *et al.* 2011). It is important for the breeder to test each genotype or plant material for sensitivity to a specific mutagen treatment to ensure induction of useful mutations without damaging the plant.

A sweet potato mutant population was successfully generated using acute gamma irradiation on *in vitro* nodal cuttings for the South African breeding programme. Approximately 8 207 mutant plants were developed from the three cultivars after four to five generations of propagation. After the first morphological screening procedure in the glasshouse, 410 mutant plants were identified with clear phenotypic changes visible to the naked eye. Changes included chlorophyll variegated leaves, change in leaf shape from triangular to lobed, fused veins, change in abaxial vein pigmentation, thin twining vines, leaf curl and root flesh colour change from cream to pale orange – yellow. Of these selected mutants, 144 were from Ndou, 188 from Monate and 78 from Mokone. A generally low mutation frequency of 4.99% was observed from the initial morphological screening as observed by other researchers (Jain 2005; Ceballos *et al.* 2008; Mehlo *et al.* 2013). To increase selection efficiency, a breeder should adopt other genetic screening procedures to identify useful mutations that could not be expressed phenotypically.

Mutants derived from Ndou were subjected to a quick vegetative drought screening procedure in the glasshouse to identify promising mutants with water stress adaptation. There was variation in results obtained from heat and drought tolerance experiment and those from drought tolerance experiment. Thirteen mutant lines (M26, M32, M37, M70, M23, M64, M45, M2, M63, M33, M5, M31, M3) were identified with overall significantly improved heat and drought tolerance compared to the control cultivar Ndou. No mutant line with significantly improved drought tolerance was identified. Mutant lines M32 and M26 were significantly more sensitive to drought than the control Ndou. Mutants with improved drought tolerance will be evaluated further in drought screening programmes.

To evaluate the effects of gamma ray mutagenesis on agronomic traits (root yield, dry mass content and flesh colour), mutant lines were planted in two field trials during 2012 and 2013 seasons respectively. Two mutant lines M224 and M6 had a visible root flesh colour change from cream (control) to pale orange-yellow. These mutants had a desirable flesh colour change, but the yields were significantly lower than that of the control Ndou. Although induced mutations can add a new desirable trait in a genotype, it might also cause undesirable mutations in several segments of the genome (Harding and Mohammad 2009) and therefore it is important to evaluate mutant plants for all important traits before selection.

Gamma irradiation in sweet potato resulted in significantly improved total root yields in two mutant lines M95 and M96 indicating the potential of this technique for yield improvement and consequently increasing food production. Improved marketable yield in M96 is important in quality and has a potential in increasing the economic value of the crop. To address quality and consumer acceptability, dry mass content was improved in two mutant lines M47 and M28 which is an important characteristic for consumption as it is often associated with good dry taste as well as long shelf life (Lebot 2009).

There was no significant improvement in Zn and Fe contents of mutant lines compared to the control. Significant improvements were observed in Mg and Mn contents resulting in 13 mutant lines (M25, M45, M47, M80, M92, M96, M99, M158, M197, M202, M212, M221 and M226) with higher Mn contents and six mutant line (M80, M100, M24, M202, M212 and M226) with higher Mg contents compared to the control Ndou. Total starch contents were higher in four mutant lines above 60% (M98, M204, M18 and M205) compared to 47.42% total starch content in Ndou.

The use of induced mutation as a tool to improve important traits in sweet potato has potential and could result in elite mutant varieties with increased yields, enhanced nutritional and economic value. Results shown in this study are from preliminary evaluations and these mutant lines will be evaluated further for each trait to enable proper identification of useful mutations for genetic use in the breeding programme. Orange and yellow-fleshed sweet potatoes have significantly higher β-carotene contents than cream-fleshed ones. It is therefore important to quantify β-carotene contents in the orange-yellow mutants in order to determine their significance in addressing VAD. Mutants with drought tolerance and yield potential will be evaluated under rain fed conditions for drought adaptation and in multi-location trials for yields and adaptation. Samples will be taken for mineral analyses, determination of starch properties and total carotenoids contents to confirm the effect of mutation breeding on the nutritional value of sweet potato. The generated sweet potato germplasm will be used as genetic resources in the South African breeding programme to address food shortages and micronutrient deficiency.

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SUMMARY

Induced mutation was incorporated into the South African sweet potato breeding programme to improve elite cultivars for yield, drought adaptation and nutritional quality. Three cream-fleshed cultivars namely Ndou, Monate and Mokone were selected, propagated *in vitro* and subjected to gamma ray mutagenesis from a ^{60}Co source at SANBS. Radio-sensitivity tests were done on three node cuttings used as explants to determine optimum dosages for bulk irradiation. Data was subjected to regression analysis and calculated LD_{50} values were 64 Gy for Ndou, 38 Gy for Monate and 55 Gy for Mokone. Variation observed in lethal dosages highlighted the importance to test each genotype or plant material for sensitivity before mutagenic treatment.

Bulk irradiation was done at respective optimum dosages and explants were propagated *in vitro* up to M_1V_4 and M_2V_5 stages to dissolve chimeras and obtain stable mutations. Three mutant populations comprised of 8 207 mutant plants were generated. These mutant plants were screened in the glasshouse for phenotypic/morphological changes visible to the naked eye. Mutant plants with changes in leaf shape, vine colour, fused veins, abaxial vein pigmentation, chlorophyll variegation on leaves and root flesh colour from cream to pale orange/yellow, were observed. After screening, 410 mutant plants (4.99%) with phenotypic changes were identified from the generated mutant populations.

Mutant plants/lines derived from the cultivar Ndou were further subjected to vegetative drought and heat tolerance screening in the glasshouse. Two experiments were conducted and these mutant lines were evaluated for drought tolerance and drought and heat tolerance respectively. Thirteen mutant lines with improved drought and heat tolerance, when compared to Ndou, were identified from the first experiment. Further drought screening procedures will be conducted to confirm these results.

Field evaluation trials were established to evaluate Ndou mutant lines. These were first evaluated in an initial evaluation trial at Lwamondo using single plants established from seedlings in a non-balanced completely randomised design. Mutant lines with three replicates each were harvested, data was collected on root yield and samples were taken and freeze dried for mineral and total starch content analyses. No mutant line had significantly improved root yields compared to Ndou. Thirteen mutant lines with significantly improved Mn and six mutant lines with significantly improved Mg contents compared to Ndou were identified. Total starch contents were significantly higher in four

mutant lines than that of Ndou. Non-significant variations were observed in Zn and Fe contents between mutant lines and the control.

Promising mutant lines were further identified and evaluated in a replicated preliminary yield evaluation trial at Towoomba. Top cuttings were used to establish the trial. Data was collected on marketable yield, unmarketable yield, total yield, root-flesh colour and dry mass content. Two mutant lines, M96 and M95, had improved total yields of 33.01 t ha^{-1} and 30.02 t ha^{-1} respectively compared to Ndou with 22.96 t ha^{-1}. Dry mass contents were also improved in two mutant lines M47 (30.33%) and M28 (29.38%) compared to the control Ndou (27.00%). Root flesh colour changes were identified phenotypically in M224 and M6 with changes from cream to pale orange/yellow.

All mutant lines will be subjected to advanced yield and nutrient evaluations including β-carotene quantification to identify mutant lines with improved yield, drought adaptation and enhanced nutritional contents to address food security and micronutrient deficiency in SA.

Keywords: *Ipomoea batatas,* gamma ray, radio-sensitivity tests, lethal dosage, yield, dry mass, flesh colour, nutrient deficiency, mineral content

OPSOMMING

Geïnduseerde mutante is in die Suid-Afrikaanse soetpatat-teelprogram geïnkorporeer om elite-kultivars te verbeter ten opsigte van opbrengs, droogte aanpasbaarheid en voedingskwaliteit. Drie roomvleiskleurige kultivars naamlik Ndou, Monate en Mokone is geselekteer, *in vitro* voortgeplant en aan gamma radiasie, vanaf 'n ^{60}Co bron van die SANBS, blootgestel. Stralings-sensitiewe toetse is op drie-stingelknoop-steggies gedoen om vas te stel wat die optimum dosering vir massa bestraling moet wees. Data is onderwerp aan regressie ontledings en die LD_{50} waarde is bepaal; dit was 64 Gy vir Ndou, 38 Gy vir Monate en 55 Gy vir Mokone. Variasie in die dodelike dosis wat waargeneem is beklemtoon die belangrikheid om genotipiese- en plant materiaal sensitiwiteit te toets vòòr die mutasie-behandeling.

Massa bestraling is by die onderskeie optimum doserings gedoen en plantjies is deur weefselkultuur ontwikkel tot op M_1V_4 en M_2V_5 stadium om moontlike chimeras te verwyder en 'n stabiele mutant te kry. Drie mutante populasies bestaande uit 8 207 gemuteerde plante is gegenereer. Die gemuteerde plante is met die blote oog, in die glashuis, vir fenotipiese/morfologiese veranderinge ondersoek. Mutante met veranderde blaarvorm, aarkleur, versmelte are, abaksiaal-aar pigmentasie, veelkleurige cholorfil vlekke op die blare en wortelvleiskleur vanaf room tot lig oranje/geel is waargeneem. Daar is 410 gemuteerdelyne (4.99%) met fenotipiese veranderinge geïdentifiseer vanuit die mutante populasie.

Gemuteerde plante/lyne afkomstig vanaf die kultivar Ndou is verder blootgestel aan vegetatiewe droogte en hitte verdraagsaamheids-evaluasies in die glashuis. Twee eksperimente is gedoen en die gemuteerde lyne is geëvalueer vir droogte verdraagsaamheid as ook vir droogte en hitte verdraagsaamheid onderskeidelik. Dertien gemuteerde lyne met verbeterde droogte en hitte verdraagsaamheid in vergelyking met Ndou is geïdentifiseer uit die eerste eksperiment. Verdere droogte-evaluasieprosedures sal nog uitgevoer moet word om die resultate te bevestig.

Veld-evaluasie proewe is gevestig om die Ndou gemuteerde lyne te evalueer. Hierdie lyne is aanvanklike in 'n proef in Lwamondo geëvalueer, enkel plante is van saailinge af gevestig en in 'n ongebalenseerde volledig ewekansige-ontwerp uitgelê. Gemuteerde lyne met drie herhalings elk is geoes, data op wortelopbrengs is versamel en monsters is geneem en gevriesdroog vir minerale en totale stysel bepalings. Geen gemuteerde lyne het 'n betekenisvolle verbetering vir wortel-opbrengs in vergelyking met Ndou getoon nie.

Dertien gemuteerde lyne met betekenisvolle verbeterde Mn en ses gemuteerde lyne met betekenisvol verbeterde Mg in vergelyking met Ndou is geïdentifiseer. Totale stysel inhoud was betekenisvol hoër in vier gemuteerde lyne in vergelyking met Ndou. Geen betekenisvolle variasie is waargeneem in Zn en Fe inhoud tussen die gemuteerde lyne en die kontrole nie.

Belowende gemuteerde lyne is verder geïdentifiseer en geëvalueer in ‘n gerepliseerde voorlopige opbrengsevaluasie proef by Towoomba. Top steggies is gebruik om die proef te vestig. Data is ingesamel vir bemarkbare opbrengs, onbemarkbare opbrengs, totale opbrengs, wortelvleiskleur en droëmassa inhoud. Twee gemuteerde lyne, M96 en M95, het verbeterde totale opbrengste van 33.01 t ha^{-1} en 30.02 t ha^{-1} onderskeidelik gelewer in vergelyking met Ndou se 22.96 t ha^{-1}. Droëmassa-inhoud het ook verbeter in twee gemuteerde lyne M47 (30.33%) en M28 (29.38%), in vergelyking met die kontrole Ndou (27.00%). Wortelvleis-kleur veranderings is fenotipies geïdentifiseer vir M224 en M6 met verandering van room to lig oranje/geel.

Alle gemuteerde lyne sal getoets word in ‘n gevorderde opbrengs- en voedings-evaluasieproef wat β-karoteen kwantifisering insluit om gemuteerde lyne te identifiseer met verbeterde opbrengs, droogte aanpasbaarheid en verhoogde voedingswaarde om voedsel veiligheid en mikrovoedingstekorte in SA aan te spreek.

Keywords: *Ipomoea batatas,* gamma bestraling, stralings-sensitiewe toetse, dodelike doses, opbrengs, droëmassa-inhoud, vleiskleur, voedingstekorte, mineraal inhoud