Participatory selection of traditional leafy vegetables and variation in morphology and mineral content of colchicine-treated *Corchorus olitorius* plants in northern KwaZulu-Natal, South Africa

by

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DECLARATION

The work described in this dissertation was carried out in the Department of Agriculture at the University of Zululand, KwaDlangezwa. These studies have not otherwise been submitted in any form for any degree or diploma at any University. Where use has been made of the work of others, it is duly acknowledged in the text.

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I certify that the above statement is correct.

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ABSTRACT

Traditional leafy vegetables (TLVs) are mostly wild-collected, nutrient-rich herbaceous plants whose leaves, shoot tips, flowers and fruits are consumed as vegetables. They are well-adapted to harsh environmental conditions and grow in soils with limited fertility. This study focused on TLVs that are utilized by a rural community of the KwaMbonambi area of northern KwaZulu-Natal, South-Africa.

A survey was conducted to identify the most preferred TLV's in this study area and to determine factors affecting their consumption and cultivation. In a total of 19 recorded TLV species, Amaranthus hybridus, A. spinosus, A. thunbergii, Bidens biternata and Corchorus olitorius, were the most preferred in the area based primarily on their appealing taste. Most TLVs were collected from the wild mainly by females, during the rainy summer season, and were consumed only once a week depending on their availability. The cultivation of TLVs was very rare because poor seed quality restricted their cultivation.

Participatory variety selection was done with the KwaMbonambi community of the study to evaluate 15 Amaranthus genotypes. Amaranthus was selected because of its popularity as a TLV in the area. During the participatory variety selection, mild-tasting and high-yielding genotypes were the most preferred. Genotypes ACAT seed fair, Tanzania and AMES-22680 were the most preferred in terms of the above traits.

Induced mutation through colchicine has been successful in many crop species but least applied in traditional leafy vegetables. The effect of colchicine treatment on 15 morphological traits and 19 leaf minerals were therefore investigated in *Corchorus olitorious. The evaluated colchicine treatments showed significant ($P < 0.05$) differences among them in both morphological and nutritional traits evaluated. Of all morphological traits measured, only the leaf chlorophyll content (LCC) of the 0.025 g l^{-1} colchicine treatment for two hours was significantly higher than the control. The trend for mineral nutrient content was similar with the highest values for most nutrients corresponding with 0.025 g l^{-1} of colchicine for two hours treatment.*

The pattern of diversity among treatments was determined by multivariate analysis procedures. For morphological traits, the first principal component (PC) accounted for 72.94% of the total variance and all morphological traits except the stem girth were the main traits that contributed positively to the variation. Whereas for mineral nutrient content the first component accounted for 68.37% of the total variation, and were mainly defined positively by N, P, K, Ca, Mg, Al, Zn and Fe. Relatively high levels of dissimilarity were observed among plants of the different treatments. Plants from treatments with low colchicine concentrations and shorter treatment durations were generally similar in all measured traits, but different from plants from treatments of high colchicine concentrations and longer treatment durations. This indicates potential for an improvement of the crop through colchicine treatment and thereby contributing to food security and balanced diets in rural households in South Africa.

CONFERENCE PRESENTATION

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ABBREVIATIONS

%:	Percent
Al:	Aluminium
Anova:	Analysis of variance
ARC-VOP:	Agricultural Research Council-Vegetable and Ornamental Plants
Ca:	Calcium
CC:	Colchicine concentration
Cm:	Centimetre
Cm^2:	Centimetre squared
Cu:	Copper
DAT:	Days after transplanting
Fe:	Iron
g l$^{-1:}$	Grams per litre
GLM:	Generalized linear model
h:	Hours
HSD:	Honest significant difference
K:	Potassium
LA:	Leaf area
LAN:	Lime (stone) ammonium nitrate
LCC:	Leaf chlorophyll content
mg kg^{-1}:	Milligram per kilogram
Mg:	Magnesium
Mn:	Manganese
MS:	Mean score
N:	Nitrogen
Na:	Sodium
NB:	Number of branches
NL:	Number of leaves
NP/P:	Number of pods per plant
NS:	Not significant
P:	Phosphorus
PC1:	First principal component
PC2:	Second principal component

PCA:	Principal component analysis
PH:	Plant height
PPB:	Participatory plant breeding
PRA:	Participatory rural appraisal
PTL:	Petiole length
PVS:	Participatory variety selection
RDL:	Radicle length
SA:	South Africa
SAS:	Statistical analysis system
SG:	Seed germination percentage
SPSS:	Statistical package for social sciences
STG:	Stem girth
TLVs:	Traditional leafy vegetables
TS:	Traits
UPGMA:	Unweighted pair group method of arithmetic means
USA:	United States of America
Zn:	Zinc

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CHAPTER 1

INTRODUCTION

The northern part of KwaZulu-Natal possesses a high diversity of traditional leafy vegetables (TLVs) which are extensively consumed in rural communities in particular (Ntuli 2013). Traditional leafy vegetables refer to the semi-domesticated plant species whose different plant parts are utilised as a vegetable (Molina et al. 2014). In South Africa, they are normally called imifino or morogo (Mavengahama 2013). They can either be cultivated as domesticated crops from their wild progenitors or grow as weeds among other crops (Langat 2014). They can also occur naturally as weeds in grasslands, forests, along the roads, footpaths as well as in wet areas (Ntuli 2013). However, a large number of these species occur in the wild (Njume et al. 2014) during the rainy seasons (Jansen van Rensburg et al. 2007).

Plants classified as TLVs can serve both for food and medicinal purposes (Langat 2014). They are a source of both macro and micronutrients (Lewu and Mavengahama 2010) including nitrogen, potassium, calcium, iron, manganese, zinc, copper, and magnesium, as well as amino acids and other hormone precursors (Flyman and Afolayan 2006). In spite of their high nutrient content their cultivation rate is low and their consumption has declined in many parts of the world (Modi 2007; Lewu and Mavengahama 2010). Preference and gender differences (Vorster et al. 2007), unfamiliar taste (Modi et al. 2006), small leaf size (Mavengahama 2013) and seasonal availability of the TLVs (Jansen van Rensburg et al. 2007) attributed to the decline in their consumption.

Increasing genetic variability is the first step in crop improvement (Azmi et al. 2016). Colchicine treatment has been reported as one of the best tools of inducing and increasing genetic variability in some edible crops within a very short period of time (Ajayi et al. 2014). Once genetic diversity is achieved, TLV species can be subjected to a breeding programme such as participatory variety selection (PVS) that will focus on improving farmer-valued traits. Participatory variety selection (PVS) is a selection process of testing released or promising genotypes in farmer’s field (Getahun et al. 2016). This system facilitates farmers to take active participation in selecting breeding lines or the finished varieties. Their early participation favours farmers to select varieties according to their preferences, needs and other expected characteristics (Kolech et al. 2017). Successful variety introduction and adoption using PVS has been reported in many countries, such as maize in South Africa (Chimonyo et al. 2019), *Brassica oleraceae* in Tanzania (Adeniji et al. 2010), *Triticum aestivum* in Ethiopia (Demelash et al. 2014), *Sorghum bicolor* in Malawi (Nkongolo et al. 2008) and *Phaseolus vulgaris* in Uganda (Bruno et al. 2018).

In modern plant breeding, researchers breed hybrids that are successful only under ideal conditions. As for many farmers such, environmental conditions do not match with theirs; as a result they obtain poor results from certified seeds and improved cultivars offered by the formal research system (Bhargav and Meena 2014). In addition to the issue of adaptability, farmers' preferences of a variety depend on a combination of traits like high yield, resistance to pests and diseases as well as good taste (Adeniji et al. 2010). This information remains unknown to breeders, unless other stakeholders such as farmers and community members are involved in a breeding programme.

Improved crops may be obtained by mutation induction through the use of anti-mitotic agents such as colchicine (Alam et al. 2011). Colchicine is an alkaloid obtained from seeds of *Colchicum autumnale* that belongs to the family Lilliaceae (Nura et al. 2011). This powdered anti-mitotic agent may be applied to growing tips, meristematic cells, seeds, axillary buds in its liquid form or mixed with lanoline (Kazi 2012).

Colchicine-treated plants have many desirable features, including increased size of all plant parts (Randolph 2016), increased vigour, and disease resistance (Ranney 2006), improved taste (Kazi 2015), enhanced flower colour (Ntuli 2007), increased content of secondary metabolites (Fasano 2013), increased photosynthesis (Ntuli and Zobolo 2008) as well as improved nutritional status (Randolph 2016). For instance, colchicine treatment increases leaf mineral content such as Aluminium, Calcium, Iron, Potassium, Magnesium, Nitrogen and Phosphorus in *Nicotiana alata* (El-Morsy et al. 2009) and *Cannabis sativa* (Bagheri and Mansouri 2015). However, colchicine-treated plants may show dwarfism which can be a disadvantage if tall plants are desired (Ntuli 2007).They are also often associated with high seed sterility, reduced germination with high mortality rate of the seedlings (Ntuli and Zobolo 2008).

1.1 Problem statement

South Africa is still challenged by high poverty rates especially in rural communities. Northern KwaZulu-Natal has a vast plant biodiversity, which includes nutrient rich TLV species that improve food security, particularly in rural areas. However, TLVs are still referred to as weeds and neglected for agronomic improvements that always focus on fully domesticated crops. Challenges associated with the consumption of TLVs include their seasonal nature, small consumed parts, unfavourable taste and lack of knowledge concerning their nutritional status. Colchicine treatment may offer one way of improving the size of their edible parts, organoleptic properties and nutritional content. Likewise, participatory variety selection facilitates farmers' awareness and further ensures that improved TLV constitute farmers' preferred traits.

1.2 Aim and objectives

This research was conducted to select the preferred TLVs at KwaMbonambi using participatory variety selection, and to study the effect of colchicine on the selected characteristics of the most preferred TLV. To achieve this, the following objectives were identified:

- To identify, through questionnaires the most preferred TLV species available and utilized in KwaMbonambi, as well as factors which limit consumption and cultivation of these species.
- To identify respondent's preferences for *Amaranthus* genotypes using their own selection criteria.
- To evaluate sensory acceptability of selected *Amaranthus* genotypes as based on the selection criteria of the respondents and to identify the best performing genotypes for cultivation.
- To establish a cost-effective, rapid and efficient protocol for colchicine induction and improving farmers preferred morphological traits using *C. olitorius* seeds.
- To determine leaf mineral content of colchicine-treated *C. olitorius* plants.

1.3 Research questions

- What are the most preferred traditional vegetables in KwaMbonambi? What are the limiting factors associated with their consumption and cultivation?
- What are the farmers' preferred traits for *Amaranthus* genotypes? What is the respondent's overall liking of *Amaranthus* genotypes?
- How does sensory profiling (taste, texture and aroma) affect the status of *Amaranthus* cultivation?
- What is the efficient protocol for inducing and improving farmers preferred morphological traits in *C. olitorious* plants using colchicine treatment?
- What is the effect of colchicine treatment on the nutritional status of *C. olitorious*? Do colchicine-treated *C. olitorious* plants have the ability to accumulate more leaf minerals in their tissues than their untreated counterparts? Are there significant differences in the leaf mineral content of colchicine treated and untreated *C. olitorious* plants?

1.4 Hypotheses

- H_1 = There is a wide diversity of TLVs occurring and preferred in the studied area.
- H_1 = Leaf number, leaf texture, taste, leaf colour, and shape are the major traits important in the preference of *Amaranthus* genotypes.
- H_1 = Colchicine-treated *C. olitorius* plants differ morphologically among themselves and with their untreated counterparts.
- H_1 = Colchicine treatment increases leaf mineral content of *C. olitorius* plants.

1.5 Structure of the thesis

Chapter 1 contains the general introduction, problem statement, research objectives, research questions and hypotheses. **Chapter 2** reviews literature that is common to all the sub-studies of this broad research.

Chapter 3 deals with the survey of identifying traditional leafy vegetables preferred at KwaMbonambi, it also reports on the various factors affecting the consumption and cultivation of TLVs. **Chapter 4** focuses on the participatory variety selection which established farmer's preference criteria for the diversity of TLVs occurring in the area. Chapters three, four, five and six have their own introduction, materials and methods, results and discussion.

Chapter 5 Involves the colchicine seed treatment of one of the most preferred TLVs, *Corchorus olitorious* as well as evaluation of the effect of colchicine concentration and treatment duration on its morphological traits. **Chapter 6** reports on the effect of colchicine treatment on leaf mineral content of *C. olitorious* plants.

Chapter 7 presents the general conclusions and recommendations made regarding the survey on preferred traditional leafy vegetables, participatory variety selection and effects of colchicine treatment on the agronomic and nutritional traits of *C. olitorious* plants.

CHAPTER 2

LITERATURE REVIEW

2.1 Traditional leafy vegetables: uses and nutritional benefits

Traditional leafy vegetables (TLVs) are commonly herbaceous plants that are mainly harvested from the wild or that naturally emerge as weeds whose leaves, shoots, flowers, fruits, seeds and/or roots are consumed as vegetables (Jansen van Rensburg et al. 2007). They are often eaten as a relish with many carbohydrate staples (Oniango et al. 2008) and other starchy foods like sorghum and maize meal (Mavengahama 2013). These plant species may not be indigenous to a country but their association with traditional production systems has increased their popularity to local people (Orech et al. 2005). They are often wild collected plants that are rarely cultivated because people believe the plants will grow naturally (Jansen van Rensburg et al. 2007).

In African countries, particularly South Africa, the reliance on these species began 2000 years ago (Jansen van Rensburg et al. 2007) and today they are a source of nutrients for millions of people (Njume et al. 2014). The most well-known and preferred traditional leafy vegetables include *Alternanthera sessilis, Amaranthus hybridus, Amaranthus hypochondriacus, Amaranthus spinosus, Amaranthus thunbergii, Bidens pilosa, Chenopodium albu, Citrullus lanatus, Colocasia esculenta, Commelina benghalensis, Corchorus* spp., *Cucurbita maxima*; *Cucurbita pepo, Cucurbita* spp., *Galinsoga parviflora, Momordica balsamina* and *Vigna unguiculata* (Ntuli 2013).

Traditional leafy vegetables have a variety of ethnomedical uses, for instance, an infusion from *Bidens pilosa* is used for the treatment of stomach pains (Lewu and Afolayan 2009) while *Cochorus olitorius* is widely consumed for its high iron and folate content that are useful for the prevention of anaemia (Ndlovu and Afolayan 2008). In fact, many traditional leafy vegetables have been reported to possess higher nutritional content than several well-known cultivated plants (Konsam et al. 2016). Those TLVs in particular with the sour taste, like *Crotalaria brevidens* and *Solanum retroflexum*, have been reported to heal stomach-related ailments (Oniango et al. 2008). In addition, TLVs also possess properties for the management of HIV/AIDS, diabetes, heart attack, cancer and others (Darkwa and Darkwa 2013).

Traditional leafy vegetables are also usually better adapted to hostile environments (Ntuli 2013), such as in poor rainfall areas as compared to most cultivated crops (Flyman and Afolayan 2006). These crops are also resistant to pests and diseases; as a result their resistant genes can act as a source of genes for the improvement of other crops (Konsam et al. 2016). Moreover, TLVs are capable of striving in the wild and this contributes to their ability to be grown with minimum amount of production inputs when cultivated (Njume et al. 2014). They also enhance crop diversity of African agricultural systems (Berinyuy and Fontem 2011).

2.2 Constraints towards the consumption of TLVs

Despite the crucial role they play in improving food security, TLVs are still neglected (Taleni et al. 2012) and underutilized in South Africa and in many parts of the world (Njume et al. 2014) as they are categorised as old-fashioned, poverty food, female food and weeds by most young people, urban dwellers and educated people (Oniango et al. 2008; Taleni et al. 2012).

TLVs are also seasonal in growth (Faber et al. 2010). This becomes a challenge to most underprivileged rural households who tend to become entirely reliant on these TLVs for food (Mavengahama 2013). However, there is an exception for black jack (*Bidens pilosa*) (Faber et al. 2010) and others that occur on irrigated fields since they normally occur in winter when others are not available (Lewu and Mavengahama 2010). When available, their small leaf sizes reduce their harvesting frequency resulting in the decline of their consumption (Jansen van Rensburg et al. 2014). Traditional leafy vegetables also tend to be low yielding when compared with fully domesticated leafy vegetables like spinach and cabbage (Mavengahama 2013). It was also revealed that they have low shelf life and unfamiliar taste as compared to most improved leafy vegetables (Taleni and Goduka 2013).

2.3 Participatory variety selection

Participatory Variety Selection (PVS) is a breeding approach that brings breeders, social scientists, farmers and extension personnel together in a field setting in order to prioritize and target traits of importance (Kolech et al. 2017). Unlike participatory plant breeding (PPB) that takes longer as several years elapse before varieties can be obtained from the breeding materials that are created; PVS relies on already existing varieties (Nkongolo et al. 2008). The PVS is important in understanding farmers' selection criteria, raising awareness, and facilitating adoption of improved varieties (Chimonyo et al. 2019). It also helps to identify and assess traits that are important to small scale farmers (Getahun et al 2016). During PVS, information is gathered on farmers' variety preferences, which helps in narrowing down the possible varieties to be introduced in a community (Bruno et al. 2018). This involves surveys; transect walks, focus group discussions and other basic tools of social science to evaluate the needs of farmers (Kucek et al. 2015).

Most breeding experiments suffer from the disadvantage that the major stakeholders are not involved in the selection and development of the varieties (Bhargav and Meena 2014; Getahun et al. 2016). Therefore, participatory varietal selection is a way to overcome the limitations of conventional breeding by offering farmers the possibility to choose, in their own environment, the varieties that better suit their needs and conditions (Getahun et al. 2016). In this model, farmers monitor the growth of the plants from the seedling to maturity in terms of drought tolerance, disease resistance, yield, vigour, maturity period and many other important attributes depending on their selection criteria (Kiiza et al. 2012). For the majority of leafy vegetable crops, the combination of horticultural traits like leaf number, leaf texture, organoleptic taste, leaf colour and shape have been reported to be the major selection criteria used for the farmers' choice of a particular leafy vegetable (Adeniji and Aloyce 2013). Previous PVS studies reported that organoleptic properties, mainly taste can be used as the basis for value judgement and became criteria against which the value for a range of species could be evaluated (Konsam et al. 2016). This is the case because ethnicity and gender had been reported to have an effect on the use and preferences of the different traditional leafy vegetables (Taleni et al. 2012; Jansen van Rensburg et al. 2014), with men preferring the more bitter taste and women and children preferring the milder taste (Vorster et al. 2007; Ntuli 2013).

2.4 Colchicine treatment

Mutagenesis is a tool in plant breeding for creation of genetic variability for further selection, hybridization and production of raw materials for genetic improvement of economically important crops (Olorunmaiye et al. 2019). Mutations may either arise spontaneously or chemically induced using mutagens. Since the natural mutation rate is usually low and may be difficult to exploit for breeding, induced mutations using chemical mutagen such as colchicine are imperative (Javadian et al. 2017; Srivastava et al. 2018). Colchicine ($C_{22}H_{25}O_6N$) is a poisonous alkaloid extracted from seeds and bulbs of *Colchicum autumnale* and *C. luteum* (Nura et al. 2011). It is one of the most commonly used chemical mutagens in plants due to its potency (Manzoor et al. 2019). Colchicine may be applied to growing tips, meristematic cells, seeds, axillary buds in aqueous solution or mixed with lanoline (Kazi 2012; Manzoor et al. 2019). It is regarded to be a shortcut breeding technique, which has produced new and high yielding varieties through heritable changes in genetic constitution (Ajayi et al. 2014). The optimum dose of colchicine and treatment duration mainly depends on the species and environmental conditions (Azmi et al. 2016).

The main action of colchicine is to prevent the formation of spindle fibres so that the anaphase movement of the chromosomes does not take place (Alam et al. 2011). Colchicine slows down metabolic processes and delays the separation of sister chromatids (Kazi 2012). When the daughter chromosomes finally divide, the chromosomes divide without moving towards poles due to lack of spindle fibres. As a result, they are all included in one cell and the chromosome number is doubled (El-Morsy et al. 2009; Alam et al. 2011; Kazi 2012).

2.5 Effect of colchicine treatment on plants

Changes in the genome of the colchicine-treated plants can cause them to differ from the untreated ones physiologically, morphologically (Moghe and Shiu 2014), ecologically and agronomically within one or a few generations (Ntuli 2007; Te Beest et al. 2012; Soltis et al. 2014). Colchicine treatment increases cell size which results in the enlargement of the plant parts (Ntuli and Zobolo 2008). As a result, colchicine-treated plants possess larger and thicker leaves, stems, roots, flowers, seeds and fruits which result in higher yield than their untreated progenitors (Andrea et al. 2006; Wu et al. 2015). For instance, it has been reported that colchicine-treated apples produce fruit that is almost double the size of untreated fruit (Nadler 2009). Moreover, the colchicine-treated plants tend to possess larger chlorophyll content (Alam et al. 2011), larger stomatal guard cells, pollen and increased DNA in the nucleus and seeds (Moghe and Shiu 2014).

Colchicine treatment also affects the morphology of the reproductive systems. These changes in the reproductive systems are responsible for the low self-incompatibility of some colchicine-treated species (Moghe and Shiu 2014). In flowering plants, it triggers variation in flowering time and flower number. The colchicine treatment effect on plant physiology includes increased adaptation to biotic stress, high level of heterozygosity, self-fertilization, and reduced inbreeding depression (De Storme and Mason 2014). These extra genomes due to induced mutations make them to be capable of tolerating and occupying harsher environments than their diploid progenitors (Fasano 2013). This was supported by the findings of Moghe and Shiu (2014) that changes in genomes are the ones that give colchicine-treated plants an improved water relation, gas exchange and cold tolerance. According to Acquaah (2007) and Randolph (2016) colchicine-treated plants also show high levels of nutritional status than their untreated counterparts. In a study by Bagheri and Mansouri (2015), in *Cannabis sativa*, colchicine treatment increased the amount of calcium, potassium, and phosphorus in the roots and leaves when compared with that of the untreated plants. The same study recorded a decrease in sulphur and magnesium content in the leaves of *Cannabis sativa*. In another study by El-Morsy et al. (2009), colchicine treatment increased foliar nitrogen and phosphorus content in *Nicotiana alata*. Similar studies have been conducted by Zhang et al. (2014) and Ghimire et al. (2016) in *Triticum turgidun* and *Miscanthus* × *giganteus,* respectively. Colchicine treatment as a method of improving morphological traits has been widely used with success for leafy vegetables, namely: *Corchorus olitorious* (Nura et al. 2011), *Lagenaria sphaerica* (Ntuli and Zobolo 2008) and *Spinacia oleracea* (Roughani et al. 2017). However, studies on the effects of colchicine treatment on the nutritional status of edible plants are still limited.

Regardless of all the above mentioned plant improvement, colchicine treatment is normally associated with a large number of drawbacks (Otto 2007). For instance, it is accompanied by a large number of reproductive barriers that may either occur before or after embryo formation. Some of these barriers include the inhibition of the pollen tube growth (Osabe et al. 2012), as well as high pollen abortion rates which restricts their commercial usefulness (Wu et al. 2015). Where reproduction is successful species usually have reduced fertility (Otto 2007), which is more prevalent in species with odd numbers of chromosomes (Ntuli 2007), this low fertility is largely caused by unbalanced chromosome interaction (Osabe et al. 2012). However this is not always the case because for other crops like *Hordeum vulgare*, *Secale cereale* and *Avena brevis* fertile plants were obtained (Randolph 2016). This mechanism is also responsible for the development of mixoploids which promotes chimeras in plants (Andrea et al. 2006) as well as a rapid loss of DNA which is normally encountered after colchicine treatment (Tayale and Parisod 2013). Lastly, colchicine-treated plants have been reported to have low auxin content as well as delayed times of flowering when compared to their untreated progenitors (Acquaah 2007).

2.6 Conclusion

This literature review recorded wide biodiversity of TLV species that are of remarkable nutritious qualities like macro and micronutrients, minerals and vitamins. Despite their high nutrient content, large quantities of TLVs are still obtained by collection in the wild rather than by cultivation. Their consumption is also declining due to people's negative perceptions; varying preferences and seasonal availability. It has been shown that the participatory plant breeding technique remains the best tool to identify farmer-preferred traits that breeding programmes should focus on. Likewise, mutagenesis through colchicine treatment is reported to cause higher morphological and molecular mutation than other mutagenic substances; thus improving crop yield within a very short period of time.

The following chapters record TLVs that are preferred in KwaMbonambi, northern KwaZulu-Natal and determine the respondents' overall liking of the most preferred genotypes. The effect of colchicine treatment on agronomic and nutritional traits of the most preferred TLV, *Corchorus olitorious*, are also reported.

CHAPTER 3

Preferred traditional leafy vegetables in the KwaMbonambi area, northern KwaZulu-Natal, South Africa

Abstract

Traditional leafy vegetables (TLVs) are mostly herbaceous plants whose leaves, shoot tips, flowers and fruits are consumed as vegetables. A survey was conducted at KwaMbonambi area in the northern KwaZulu-Natal province of South Africa using a structured questionnaire. This sourced information on the consumption, preference, collection and cultivation of these leafy vegetables in the area. A total of 18 TLVs that belonged to 14 genera and 11 families were recorded. The most preferred species were: Amaranthus hybridus, A. spinosus, A. thunbergii, Bidens biternata and Corchorus olitorius. Major selection criterion for preference was on taste. TLVs were predominantly collected as weeds growing among major crops in cultivated areas, mainly during summer. Females were the primary collectors of TLVs. All species had their leaves cooked as a side dish to pap. The majority ate TLVs once a week depending on their availability. The major TLV consumption constraint was seasonal availability. A decline in the availability of TLVs in the wild was primarily caused by drought. A low percentage (23%) of respondents practiced informal cultivation of particularly Amaranthus species through seed broadcasting among main crops. Bidens pilosa, Momordica balsamina and Corchorus olitorius vegetable species were also known to possess some medicinal values.

3.1 Introduction

Traditional leafy vegetables (TLVs) are plant species whose different plant parts are consumed as vegetables (Molina et al. 2014). Such plants can serve both for food and medicinal purposes (Njume et al. 2014). Consumed plant parts include tender or mature stems, leaves, roots, flowers as well as immature and mature fruits (Jansen van Rensburg et al. 2007; Ntuli 2013). TLVs are generally consumed as a relish with staple foods such as maize meal (Matenge 2011; Mavengahama 2013).

Most TLVs grow during warm and wet seasons (Langat 2014), while others grow year round in wet areas (Lewu and Mavengahama 2010; Ntuli 2013). They can either be collected from the wild or can be cultivated (Molina et al. 2014). Large quantities of various TLVs normally grow as weeds among other field crops in cultivated land (Nkomo and Kambizi 2009). They can also occur naturally as weeds in grasslands, forests, along the roads and footpaths (Ntuli 2013). TLVs are normally collected by females being assisted by children (Ngone et al. 2016). As a result, in most cases the knowledge of TLVs tends to be in the female domain (Ntuli 2013; Langat 2014; Chakravarty et al. 2016).

The consumption of TLVs is common, while their cultivation is very rare compared with fully domesticated species (Tumwet et al. 2014). Preferences and gender differences (Vorster et al. 2007), unfamiliar taste (Taleni et al. 2012), small consumed parts (Mavengahama 2013), low shelf life and seasonal availability of the TLVs (Mampholo et al. 2016) have all contributed to the decline in their consumption. In addition, most TLVs are regarded as weeds, food for the poor (Jansen van Rensburg et al. 2014); woman’s food (Nkomo and Kambizi 2009); and old-fashioned by young and urban people (Njume et al. 2014; Van der Hoeven 2014; Konsam et al. 2016). Seeds of TLVs are also associated with dormancy and the young plants are susceptible to bolting which results in low germination and yield, respectively (Mavengahama 2013).

Considering the potential of TLVs as a source of nourishment, there is a need to change the perception of particularly young and urban people regarding these crops (Lewu and Mavengahama 2010). There is also a need to select and breed for enhanced palatability (Ntuli 2013), tolerance to low winter temperatures to overcome seasonal availability as well as finding ways to establish high quality seeds (Njume et al. 2014). Therefore, the aim of this study was to identify the most preferred TLV species in KwaMbonambi, as well as factors which affect consumption and cultivation of these species.

3.2 Materials and methods

The survey was conducted at the Bhubhubhu, Ekusayeni, Mboholo and Msunduze villages of KwaMbonambi area (28°37' South, 32°04' East), of the King Cetshwayo district, northern KwaZulu-Natal Province, South Africa. Data were collected by means of a questionnaire which was compiled in English but administered in isiZulu. A pre-tested structured questionnaire was administered randomly to 100 respondents, a total which was drawn from 25 homesteads in each village. Prior to interview engagement, the purpose and nature of the study was explained to respondents and a written consent form was signed thereafter. Selection of households was done using simple random sampling. Once the household was selected, the respondent was chosen using purposive sampling in that an elderly and/or knowledgeable individual was interviewed. Both male and female respondents were grouped according to age in the following age ranges: young-age (18-34 years); middle-age (35- 54 years); and old-age (55 years and above).

During the survey, respondents listed the TLVs that they preferred with reasons. The information on: source of collection; season of collection; ecology from which they were collected; collectors, plant parts consumed as well as their consumption frequency were recorded. The other information collected included various staple foods which were accompanied by the TLVs. Information on TLV quantities and cultivation status was also collected. Propagation methods, other uses apart from consumption as well as constraints towards their consumption were also recorded. Voucher specimens were prepared from identified leafy vegetables and housed at the University of Zululand Herbarium (ZULU).

3.3 Data analysis

Data were subjected using IBM SPSS (2016) version 22 software, where each questionnaire represented a case and the questions on the questionnaire represented variables and sub-variables. The simple descriptive statistics, such as frequencies and percentages were used to analyse the data and tables used to summarize the data set.

3.4 Results

3.4.1 Gender and age of interviewees

A total of 88 females and 12 males from different age groups were interviewed in this study (Table 3.1). In all villages, the majority of female interviewees were in the middle-age group followed by old-aged group and the minority in the young-aged group.

Table 3.1: Gender and age of respondents from four villages

Villages	Age group	Males	Females
Bhubhubhu	Young-age	0	3
	Middle age	1	9
	Old age	4	8
Mboholo	Young-age	0	4
	Middle age	1	11
	Old age	1	8
Msunduze	Young-age	0	1
	Middle age	2	14
	Old age	0	8
Ekusayeni	Young-age	0	3
	Middle age	2	12
	Old age	1	7
Total number of respondents		12	88

Age group: Young age, 18-34 years; Middle age, 35-54; Old age, > 55 years.

3.4.2 Taxonomy and preference of TLVs at KwaMbonambi

Eighteen traditional leafy vegetable species that belong to fourteen genera and eleven families were identified in the current study (Table 3.2). The majority of preferred species belonged to Amaranthaceae (4 species), Asteraceae (3 species), Aizoaceae (2 species), Brassicaeae (2 species), and Chenopodiaceae (2 species) families, in their descending order. The Acanthaceae, Cucurbitaceae, Solanaceae, Tiliaceae, Verbenaceae and Zygophyllaceae families were represented by only one species each. The most preferred species in their descending order were: *Amaranthus thunbergii*; *A. spinosus*; *Bidens biternata*; *A. hybridus* and *Corchorus olitorius*. These species were preferred by both females and males. *Chenopodium album* was preferred by 35% of the interviewees, who were both females and males. *Bidens pilosa*, *Momordica balsamina* and *Sonchus oleraceus* were preferred exclusively by males. However, females exclusively favoured *Alternanthera sessilis*, *Asystasia schimperi*, *Brassica carinata*, *Chenopodium murale*, *Sisymbrium thellungii*, *Solanum retroflexum*, *Tetragonia decumbens*, *T. tetragonioides* and *Tribulus terrestris*.

Reasons for preference varied among: taste; size of the consumed plant part; easy access; familiarity to the vegetable and ease of preparation. Almost 80% of respondents preferred these leafy vegetables according to the taste (Figure 1). Above 60% of the interviewees favoured these vegetables as per their local availability and ease of preparation. Local taxonomic variation was minimal (one name per vegetable species) on the majority of these vegetables (Table 3.2). However, *T. decumbens* had four local names, while *B. pilosa*, *C. album* and *T. tetragonioides* had two local names each.

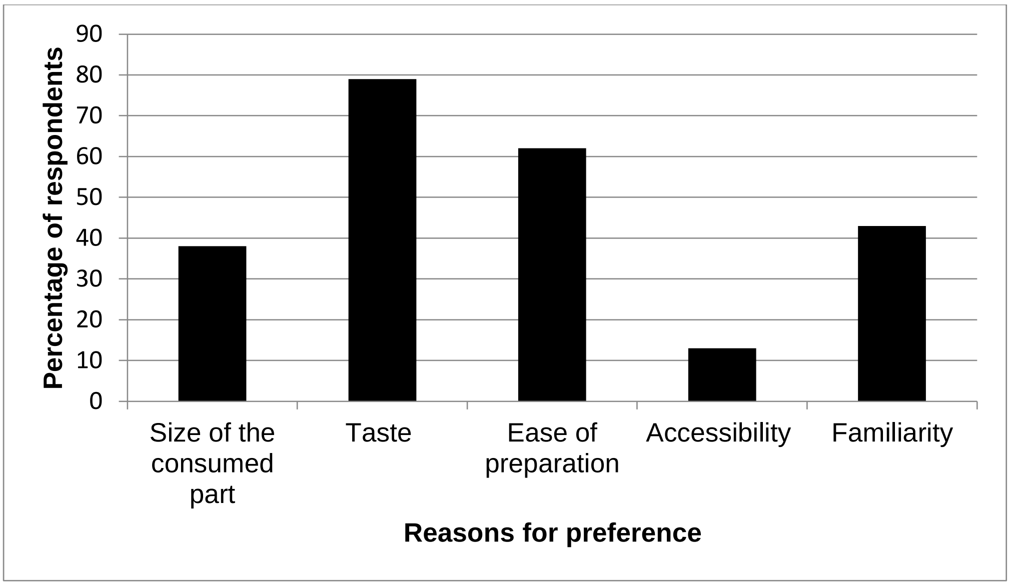


Figure 1: Reasons for preference of traditional leafy vegetables

3.4.3 Edible parts, method of preparation, sources of TLV and their seasonal availability

All vegetable species had their leaves cooked as leafy vegetables (Table 3.2). In addition, shoot tips of *A. hybridus*, *A. spinosus*, *A. thunbergii*, *B. biternata*, *B. pilosa*, *C. album* and *T. tetragonioides* were also consumed. TLVs were boiled first and then fried afterwards. This included a discard of the boiling water and its replacement with fresh water in case of bitter-tasting species such as *Bidens pilosa*. The majority (89%) respondents preferred to serve various TLVs as a side dish to supplement pap, a staple dish. The minority served TLVs as a side dish to rice, bread, amaDumbe and sweet potatoes (Table 3.3).

Collection sites for TLVs varied from cultivated land, forest, grassland, riverbank, to shrubland (Table 3.2). The majority (79%) of these vegetables were collected among crops in cultivated land. Only *A. sessilis* was collected from the riverbank. These vegetables are mostly collected in summer. However, *A. sessilis* and *B. pilosa* are collected year round, while *A. schimperi*, *B. carinata*, *T. decumbens* and *T. terrestris* are also available in spring.

Table 3.2: Taxonomy, percentage of respondents preferring each TLV, edible plant part (s), place and period of collection (n=100)

Species name	**Family name**	**IsiZulu name**	**Percentage and gender**	**Edible part(s)**	**Source**	**Season**
Alternanthera sessilis (L.) DC.	Amaranthaceae	iNtombiyaqhenya	8 (F)	L	RB	YR
Amaranthus hybridus L.	Amaranthaceae	iMbuya enkulu	75 (F, M)	L,ST	C, FR, G, SL	S
Amaranthus spinosus L.	Amaranthaceae	iMbuyabathwa	90 (F, M)	L,ST	C, FR, G, SL	S
Amaranthus thunbergii Moq.	Amaranthaceae	iMbuya encane	99 (F, M)	L,ST	C, FR, G, SL	S
Asystasia schimperi T. Anderson	Acanthaceae	iMbobela	10 (F)	L	C, FR	S, SP
Bidens biternata (Lour.) Merr.& Sherff	Asteraceae	uQadolo omhlophe	82 (F, M)	L,ST	C, FR, G	S
Bidens pilosa L.	Asteraceae	uQadolo omnyama; uCucuza	25 (M)	L,ST	C, FR, G	YR
Brassica carinata A. Braun	Brassicaeae	iKhop'shini	32 (F)	L	C, G	S, SP
Chenopodium album L.	Chenopodiaceae	iMbilikicane engababi; isiDwaba sesalukazi	35 (F, M)	L,ST	C, FR	S
Chenopodium murale L.	Chenopodiaceae	iMbilikicane ebabayo	22 (F)	L	C, FR	S
Corchorus olitorius L.	Tiliaceae	iGusha	63 (F, M)	L	C, G	S
Momordica balsamina L.	Cucurbitaceae	Intshungu	7 (M)	L	C, FR	S
Sisymbrium thellungii L.	Brassicaeae	IsiQange	45 (F)	L	C, FR	S
Solanum retroflexum Dunal	Solanaceae	umSobo	20 (F)	L	C, SL, FR	S
Sonchus oleraceus L.	Asteraceae	iKlabeklabe	4 (M)	L	C, G	S
Tetragonia decumbens Mil	Aizoaceae	uBhocobele; isKhakha somkhovu; uThambisa; uMaNtombela	10 (F)	L	C, G	S, SP
Tetragonia tetragonioides (Pallos) Kuntze	Aizoaceae	isiPinashi samaNdiya; isiPinashi seNtaba	25 (F)	L,ST	C	S
Tribulus terrestris L.	Zygophyllaceae	uNkunzana	1 (F)	L	G	S, SP

Gender: F- female; M- male. Edible part(s): L- leaves; ST- shoot tips. Source(s): B- bush; C- cultivated land; FR- forest; RB- river bank; G- grassland; SL- shrubland. Season: S- summer; SP- spring; YR-year round.

Results showed that the availability of the preferred TLVs year-round occurs mostly when they grew in permanent wet places or when the species is drought resistant. However, in this study 96% of the TLVs were collected from the intermittent wetlands. This could be the major reason for their high scarcity during the dry season (winter).

3.4.4 Collection and consumption frequency and constraints of TLVs

Collection of traditional leafy vegetables is practiced mainly by females (95%) rather than males (5%) (Table 3.3). Apparently, the majority of male respondents regarded the collection of TLVs as a females' job.

Table 3.3: Collection and consumption of TLVs

Variable	Proportion of respondents
Responsible collectors	
Females	95
Males	5
Consumption frequency	
Once a week	41
Twice a week	28
Thrice a week	15
Daily	16
Food consumed with	
Pap	89
Rice	4
Bread	3
amaDumbe	2
Sweet potatoes	2
consumption constraints	
Seasonal availability	95
Taste	12
Low shelf life	81
Smaller size of the consumed part (s)	31

Participants also mentioned that both male and female youth do not regularly collect TLVs because of the perception that it makes their nails dirty. Also, most of young people regarded the collection of TLVs as an activity that lowers their dignity, yet they participated in their consumption. The majority (41%) of respondents consumed TLVs once a week, while the minority (15%) ate them three times a week (Table 3.3). Apparently, the major reason for consumption only once a week was because, according to them, frequent intake of such vegetables leads to stomach complaints. Further, the daily (frequent) consumption is only possible when these vegetables are locally abundant, particularly during rainy season.

The major constraints toward TLV consumption were due to their seasonal availability (95%) and low shelf life (81%) (Table 3.3). All respondents were cooking the freshly-harvested vegetables and none of them have practiced drying the vegetables for a prolonged storage. In cases where large quantities of TLVs were collected for future use, these were kept uncooked in a fridge to prolong their shelf life. It was also recorded that the consumption of TLVs does not follow any regular pattern as their consumption normally increases with the availability.

3.4.5 Availability of TLVs and reason for their decline

The majority of respondents (96%) noted a decline in the availability of TLVs in the area (Table 3.4). The major reason given for the decline was drought (95%) which was also believed to restrict the consumption of various TLVs. This explained the respondents' reports on ubiquitous availability of only *Amaranthus* and *Bidens* species in the area, while some could be remembered by elderly respondents even though they are no longer found in the area. In fact according to the respondents, currently all TLVs are difficult to obtain even during summer.

Table 3.4 Wild assessment, production system and production constraints of TLVs

Variable	Proportion of respondents (%)
Quantity of TLVs in the wild	
Decreasing	96
Not changing	2
Unknown	2
Reasons for decline in the wild	
Drought	95
Overharvesting	4
Outbreak of invasive plants	1
Do you cultivate TLVs?	
No	77
Yes	23
Production system used	
Monocropping	0
Intercropping	100
Seed sowing method	
Broadcasting	100
Dribbling	0
Type of fertiliser used	
Organic	100
Inorganic	0
Production constraints of TLVs	
Lack of high quality seeds	55
Seed unavailability	6
Sole growth of fully domesticated vegetables	27
Unawareness	12

3.4.6 Production system and cultivation status of preferred TLVs

Of the 100 respondents in this survey, only 23 cultivated TLVs (Table 3.4). Out of the diverse TLV species preferred in the study area, only amaranth species were cultivated as intercrop to main cultivated crops. Strictly mature and dry seeds from the previous TLV species were broadcasted among other summer crops soon after the addition of cattle manure. Intercropping of these leafy vegetables was also facilitated by their selective weeding. The survey data further revealed that TLVs responded well in the presence and also in the absence of the organic fertilizer.

The majority of respondents (55%) identified the lack of high quality seeds as the major factor that restricted the cultivation of TLVs. For instance, one of the respondents stated that she tried some of the amaranth seeds in her garden and none of them germinated thus she was discouraged from sowing them again. As a result, in the study area the cultivation of fully domesticated leafy vegetables was more common than that of TLVs due to the fact that those seeds are always available in the market. In addition, all the elderly respondents mentioned that they felt obligated to cultivate the fully domesticated ones, even though they prefer TLVs.

3.4.7 Health and medicinal uses of some TLVs

In the KwaMbonambi area, *Bidens pilosa* was reported to treat and manage heart burn, ear infection, kidney problems, stomach cramps as well as to clean the uterus after giving birth. *Momordica balsamina, Solanum retroflexum* and *Cochorus olitorius* were known to manage diabetes, flu and lack of water in the knees, respectively. Some respondents believed that the regular consumption of TLVs promotes immunity against various diseases.

3.5 Discussion

3.5.1 Gender and age differences on the preference of TLVs

A higher percentage of females in the middle age were interviewed in different villages of KwaMbonambi area. This could have resulted because females of this age group are predominantly found at home as they take care of their young ones. This corresponds with the reports by Ntuli (2013) and Konsam et al. (2016). The responses of females from old age group were comprehensive (detailed) on the identification and uses of current TLV species and those that are no longer growing in the area. An unusual and contradictory finding was reported from the Umkhanyakude District where young males possessed more knowledge of TLVs than males of other age groups (Ntuli 2013).

3.5.2 Taxonomy and preference of TLVs

The present study showed that the people of KwaMbonambi area consume a variety of TLV species. Consumption of species within the Aizoaceae, Acanthaceae Amaranthaceae, Asteraceae, Brassicaeae, Chenopodiaceae, Cucurbitaceae, Solanaceae, Tiliaceae and Zygophyllaceae reported in the study, concurs with the reports in northern KwaZulu-Natal (Ntuli 2013) and other rural areas of South Africa (Jansen van Rensburg et al. 2007; Njume et al. 2014). Local taxonomy of these vegetables was predominantly one name per species, except for four out of nineteen documented species with more than one name. These results contradict with the reports by Lewu and Mavengahama (2010), who reported a wide variation in local taxonomy of leafy vegetable species. However, this minimum diversity in local naming might be a result of the small surveyed area of KwaMbonambi.

Amaranthus thunbergii; *A. spinosus*; *Bidens biternata*; *A. hybridus* and *Corchorus olitorius* were the most preferred leafy vegetables in this study. The first four of these vegetables were earlier rated as the most popular TLVs in northern KwaZulu-Natal (Ntuli 2013). Also, the preferred taste and ubiquitous availability of several *Amaranthus* species in most parts of South Africa (Njume et al. 2014; Van der Hoeven 2014) might explain their preference as leafy vegetables in the KwaMbonambi area. Their preference could also have been due to the effect of their choice by both males and females in the current study. *Amaranthus thunbergii* was also regarded as the tastiest leafy vegetable of all preferred species, in spite of its smaller leaves and scarcity compared to large-leaved and abundant *A*. *spinosus* and *A. hybridus* species. In Limpopo, *A. hybridus* and *A. thunbergii* were the most commonly eaten TLVs (Faber et al. 2007). Good taste was also an important trait for selecting *C. olitorius, T. decumbens and A. schimperi* in the current study. However, other respondents complained about their low leaf yield which makes their collection difficult. Apparently, the tedious collection of TLVs with small leaves has a negative impact on their use, thus most people prefer commercial vegetables with bigger leaves (Mavengahama 2013). *Amaranthus spinosus* was the second for taste preference, but its spines make its collection difficult. Similarly, in Kenya the vegetable use of *A. spinosus* is declining because of spines which restrict its harvest and sorting (Keding et al. 2007). Therefore, there is a need to select and breed for a broad-leaved *A. thunbergii* and spine free *A. spinosus*.

The bitter tasting *M. balsamina* and *B. pilosa* were mainly favoured in the present study for their medicinal value and less for food consumption. Further, bad smelling *Chenopodium album* and *C. murale* were mainly preferred for their soft texture. Most respondents prefer to cook various TLVs together to overcome either the bitterness or toughness that is often associated with TLVs. Langat (2014) and Van der Hoeven (2014) also reported that TLVs are mixed to overcome bitterness. Such practise is also believed to diversify the diet especially among the undernourished rural populations in most developing countries (Chauhan et al. 2014). Species such as *Brassica carinata, Sisymbrium thellungii and Tetragonia tetragonioides* were largely preferred in the current study because of their large consumed parts, appealing taste and pleasant aroma.

The majority of TLVs in the present study were preferred by females in all age groups, either exclusively or together with males. This was probably the case because females were responsible for cooking and maintaining household food security. In northern KwaZulu-Natal (Ntuli 2013) and India (Konsam et al. 2016), the most knowledgeable respondents on TLVs were females in their middle age. Sithole et al. (2011) and Ngone et al. (2016) also reported that old and young females in general possess more knowledge of TLVs since they are actively involved in their collection and cooking.

In the current study, the bitter tasting *B. pilosa, C. murale* and *M. balsamina* were mainly preferred by males as compared to females and the youth. A similar trend was documented where in northern parts of South Africa males appreciated bitter tasting TLVs (Jansen van Rensburg et al. 2007). Njume et al. (2014) also reported the bitter tasting *B. pilosa* as mostly appreciated by males.

This study showed that the important traits for TLVs preference were mostly taste, availability, and size of consumed plant part(s) in KwaMbonambi. This concurs with the report that the usage of specific TLV species is often influenced by factors such as availability, ease of preparation or collection, taste, consistency and appearance (Jansen van Rensburg et al. 2014).

3.5.3 Edible parts, method of preparation, sources and seasonal availability of TLVs

In all TLVs, the leaves, including the shoot tips in some species, were consumed as leafy vegetables in this study. This consumption of leaves and shoots concurs with the findings of Van der Hoeven (2014) and Ngone et al. (2016). The majority consumed TLVs as a side dish to pap while the minority fraction ate them with rice, amaDumbe and/or sweet potatoes. The consumption of TLVs as a relish that accompanies pap in South Africa was reported earlier by Mavengahama (2013).

Almost all leafy vegetables in the present study were collected among crops cultivated in intermittent wetlands. However, these were also found in areas such forest, grassland, shrubland and riverbank. The common occurrence of TLVs as weeds among cultivated crops was earlier reported by Jansen van Rensburg et al. (2007) and Vorster et al. (2007). TLVs in the current study were mostly available during rainy season in summer and some in spring. The exception was the drought resistant *B. pilosa,* as well as *A. sessilis* that grow on river banks, which were both available year round. Faber et al. (2010) also reported that *B. pilosa* can be harvested even in winter when other TLVs are no longer available. There is therefore a need to breed TLVs for resistance to drought and cold to overcome their seasonal availability shortfall.

3.5.4 Collection and consumption frequency and constraints of TLVs

In KwaMbonambi, TLVs are predominantly collected by females. Males consider such practice as the females' responsibility while the youth perceives such vegetable collection as lowering their dignity although they do consume them. This supports findings by Legwaila et al. (2011) and Njume et al. (2014) who reported that leafy vegetable harvesting in most developing countries is done by women and children.

The infrequent (once a week) and small quantity of consumption of TLVs to reduce the possibility of stomach complaints was reported in this study. It is known that frequent and large quantity intake of phytochemicals containing TLVs such as *C. olitorius* usually results in toxicity problems (Matenge 2011; Tumwet et al. 2014). Low TLV consumption frequency might have been caused by their seasonal availability, as some respondents were willing to eat them regularly depending on their availability. The findings of another survey conducted in Western Kenya also indicated an increase in the consumption of TLVs during the rainy seasons when they are locally abundant (Tumwet et al. 2014). Frequent collection and consumption (daily and three times a week) of these vegetables might have been enhanced by low-income status of several rural-based families in the current study. According to Taruvinga and Nengovhela (2015), the consumption of various TLVs is determined by poverty status because those who earn more tend to rarely consume these TLVs. Also, the unavailability of these vegetables results in certain vulnerable families being exposed to hunger (Lewu and Mavengahama 2010).

Factors that limited TLV consumption included their seasonal availability, low shelf life, smaller size of consumed plant part(s) and unpleasant taste, in a descending order. Communities had to store most of the recently collected TLVs in fridges to overcome the problem of low shelf life. Lewu and Mavengahama (2010) also recorded perishability as a major constraint with the storage, distribution and even the marketing of TLVs. The time-consuming collection and preparation of small leaved vegetable species hampered their consumption in the study area. Less traditionally orientated females were reported to prefer less time-consuming tinned food which slowly shifts their interest from TLVs that require tedious collection and processing (Tumwet et al. 2014). Several studies reported on the poor taste of TLVs compared with fully domesticated vegetables as a major reason for the decline in their consumption (Medoua and Oldewage-Theron 2014; Sowunmi 2015; Taruvinga and Nengovhela 2015; Mampholo et al. 2016; Ngone et al. 2016), which supports the findings of the current study.

The majority of respondents declared drought as the main reason for traditional leafy vegetable population decline in the study area. Similar findings were reported earlier in KwaZulu-Natal, Mpumalanga and Limpopo provinces of South Africa (Vorster et al. 2008) and also in Western Kenya (Tumwet et al. 2014). Reports from communities of Manipur, northeast India, indicated that species disappearance in the wild results in loss of their knowledge by the communities (Konsam et al. 2016).

3.5.5 Production system and cultivation status of preferred TLVs

Only 23% of the respondents informally cultivated TLVs in KwaMbonambi. Even though the commercial value and role in food security of TLVs has been highlighted in other studies (Langat 2014; Van der Hoeven 2014; Konsam et al. 2016), their production is still very low in this area. Respondents mentioned that only *Amaranthus* species were cultivated through selective weeding and/or broadcasting as intercrop among cultivated species. Several studies support this notion that in South Africa, many TLVs are still obtained by collecting rather than by cultivation and if cultivated the number of species is limited and cultivation occurs only on a small portion of land (Keding et al. 2007; Berinyuy and Fontem 2011; Njume et al. 2014; Tumwet et al. 2014; Ngone et al. 2016). Research regarding potential cultivation of traditional leafy vegetables such as *Amaranthus hybridus* and *A. tricolor* (Ribeiro et al. 2017); *A. cruentus*, *Corchorus olitorius* and *Vigna unguiculata* (Maseko et al. 2015; Mavengahama et al. 2016) has been initiated in South Africa.

In the current research, the fully domesticated leafy vegetables were frequently consumed because they were the ones which were cultivated. Taruvinga and Nengovhela (2015) reported that the consumption of various TLVs was positively correlated to their production. The lack of high quality seeds was the major constraint towards production of TLVs in this area and it was also discouraging the preservation of seeds from the previous season. Some studies have also shown that the seeds from wild species are generally dormant which discourages their production (Etèka et al. 2010; Mavengahama 2013). Similar studies also emphasized the importance of finding ways to establish seed systems for various TLV species, which can lay a foundation for their commercial production.

Most people focus on planting crop species with a pleasant taste, which are frequently consumed, and has a high yield; and thus has good market value (Konsam et al. 2016). Therefore there is a need to improve traits such as taste, growth and yield of TLVs for purposes of future cultivation and commercialisation.

3.5.6 Health and medicinal uses of TLVs

The bitter tasting *B. pilosa* and *M. balsamina* were among the reported TLVs that possess medicinal values in the current study. Ntuli (2013) reported that the bitter TLVs are associated with medicinal use. Older people of KwaMbonambi reported that the consumption of TLVs helped them to cope with several diseases. It was also reported by Lewu and Afolayan (2009) that most TLVs are believed to have health benefits which are still highly exploited at household level. There is also a belief that in South Africa the rural population consuming a traditional diet rich in green leafy vegetables are less affected by diseases such as coronary heart disease, hypertension, strokes and type 2 diabetes; in comparison to those that follow a modern, urban diet (Matenge 2011). Therefore the multipurpose use of these plants can be an important reason for their nourishment and health (Konsam et al. 2016).

Based on the present study, *Bidens pilosa* was reported to treat ear ache, kidney failure, heart burn, stomach cramps, and uterus cleansing. Similar findings of the *B. pilosa* infusions to reduce stomach complaints were earlier reported by Lewu and Afolayan (2009). In the current study *C. olitorius* was reported to help in the lack of water in the knees. However, Nyadanu et al. (2017) regarded it as a good source of vitamin A especially in children. *Solanum retroflexum* was reported in the current study to treat coughs and improve appetite. In Narok, *S. retroflexum*, is used as a remedy to prevent and manage hypertension, skin diseases and tooth decay (Langat 2014). *Momordica balsamina* was used to manage diabetes and hypertension at KwaMbonambi. Similar findings on *M. balsamina* were also reported by Mavengahama (2013).

3.5.7 Conclusion

This study reported the preference of a wide variety of traditional leafy vegetables that are all collected from the wild and cultivated fields. *Amaranthus hybridus, A. spinosus, A. thunbergii, Bidens biternata* and *Corchorus olitorius* were the most preferred TLVs. The selection of these leafy vegetables was based primarily on taste and their seasonal availability. These vegetables were mainly collected among cultivated crops during rainy seasons such as spring and summer. The major constraint for their cultivation was dormancy and low seed quality. The current study mentioned for the first time the use of rice, amaDumbe and sweet potatoes as the staple food accompanied by TLVs as a side dish was the first report. The findings of this chapter led to further investigations on the respondents' preferences for *Amaranthus* genotypes using their own selection criteria, as presented in the following chapter.

CHAPTER 4

Participatory selection of *Amaranthus* genotypes in the KwaMbonambi area

Abstract

The study investigated respondent's overall preference of 15 Amaranthus genotypes from ARC-VOP genebank using participatory variety selection (PVS). Seedlings of each genotype were planted in separate 10 x 10 m plots in a rural area near KwaMbonambi in the KwaZulu-Natal province. Fourteen respondents (ten females and four males) identified their preferred traits through direct observations and group discussions. When the plants were ready for evaluation, the genotypes were ranked by the respondents according to their preferred traits using score sheets designed in a 4-point Likert scale. They identified mild taste, bigger and numerous leaves, soft texture, and longer shelf life as important traits for preferred Amaranthus genotypes. Genotypes ACAT seed fair; Tanzania and AMES-22680 were the most preferred in terms of the chosen traits. The genotypes are thus recommended for multi-environment testing, seed multiplication, and promotion of cultivation in KwaMbonambi.

4.1 Introduction

Participatory variety selection (PVS) is a selection process that involves a close farmer-researcher collaboration in testing released or promising genotypes in farmer's field (Getahun et al. 2016). This approach helps reduce the amount of time required to move varieties to the farmers' field and determine varieties that farmers want to grow, learn traits that farmers value and determine the gender differences in varietal selection criteria (Bruno et al. 2018). It also overcome the disadvantage imposed by modern plant breeding where improved varieties are selected in favourable environments that do not represent the actual conditions on which a particular plant will be later subjected to (Migliorini et al. 2016).

In PVS, farmers are provided with a basket of genotypes for matching with their own selection criteria (Chimonyo et al. 2019). This offers farmers the possibility to choose, in their own environment, the varieties that better suit their needs and conditions (Getahun et al. 2016). It also helps to identify and assess traits that are important to small scale farmers and is especially subjective traits such as taste, aroma, appearance, texture, storage quality and other culinary qualities, which are difficult to measure quantitatively (Demelash et al. 2014). Survey studies; transect walks and focus group discussions also form part of the PVS program to assist with identifying farmers' needs (Kucek et al. 2015; Nduwumuremyi et al. 2016) through participatory rural appraisal (PRA) approach.

Genus *Amaranthus* belongs to Amaranthaceae family and originated in South America (Gerrano et al. 2017). It contains more than 60 species that are often cultivated as leafy vegetables, ornamental plants and grains (Achigan-Dako et al. 2014; Mbwambo et al. 2015). The consumable parts include young leaves, shoot tips and whole seedlings that are harvested in fallow lands and fields mainly during summer (Gerrano et al. 2017). In most rural areas, the harvested *Amaranthus* is cooked and consumed as a relish with pap. Apart from consumption, all parts of the plant are also used as medicine to heal various ailments (Devi et al. 2017). For instance, *A.spinosus* L. roots are used for indigestion and to cure dysentery (Begum and Mandal 2016), headaches, and pimples (Islam and Rahman 2017), *A.viridis* L. leaves are used to treat stomach ulcers, while its shoot extract is used in case of poisonous bites and also as an emollient (Devi et al. 2017).

Amaranthus genotypes are resistant to heat, drought, diseases and pests and has high nutritional value (Mbwambo et al. 2015; Gerrano et al. 2017). Compared to cabbage, *Amaranthus* contains more vitamin A; iron and calcium, respectively (Kansiime et al. 2018). In spite of the high nutrient content, vegetable *Amaranthus* has received significantly less research attention than grain *Amaranthus* (Hilou et al. 2016). Also, in South Africa, *Amaranthus* is rarely cultivated because it is believed that it will grow spontaneously (Jansen van Rensburg et al. 2007). Only *A. hypochondriacus, A. cruentus* and *A. dubius* are commonly cultivated in Africa (Olusanya 2018).

Adeniji and Aloyce (2013) studied farmers' preferences and discovered that several traits are important in the choice of a leafy *Amaranthus*. These are aroma, leaf yield, leaf texture, taste, leaf colour, and shape. According to Hiscock et al. (2018) there are no sensory analysis studies that have been performed on the different *Amaranthus* genotypes in South Africa. Therefore, the information generated through PVS will further assist with identification of farmer-preferred traits that could be incorporated in future *Amaranthus* breeding programmes.

4.2 Materials and methods

4.2.1 Plant material

Seedlings of fifteen *Amaranthus* genotypes were supplied by the Agricultural Research Council – Vegetable and Ornamental Plants (ARC-VOP) genebank (Table 4.1). These particular *Amaranthus* genotypes were selected because they were uncharacterised accessions for consumer acceptability (Jansen van Rensburg 2018). However, some of the accessions listed in Table 4.1 were preliminary characterised for agronomic (Gerrano et al. 2015; 2017) and nutritional traits (Gerrano et al. 2019).

Table 4.1: List of *Amaranthus* genotypes evaluated in participatory genotype selection

No.	**Botanical name**	**Cultivar name**	**Origin**
1	*A. cruentus*	AMES-22680	USA
2	*A. cruentus*	Kobie	Unknown
3	*A. cruentus*	AM-fune	Tanzania
4	*A. cruentus*	Anna	Germany
5	*A. cruentus*	Arusha	Tanzania
6	*A. thunbergii*	A. *thunbergii*	SA, Kwa-Zulu Natal
7	*Amaranthus* sp.	ACAT seed fair	SA, Kwa-Zulu Natal
8	*Amaranthus* sp.	TOT 2358	Unknown
9	*Amaranthus* sp.	TOT 2266	Unknown
10	*Amaranthus* sp.	TOT 4151	Unknown
11	*Amaranthus* sp.	TOT 8789	Unknown
12	*Amaranthus* sp.	Tanzania	Tanzania
13	*A. viridis*	W6297N	USA
14	*A. tricolor*	Tricolor p462179	USA
15	*A. greazicans*	Thoyandou	SA, Limpopo

SA-South Africa; USA-United States of America.

Source: Gerrano et al. (2017).

As a result there were still many information gaps regarding their characterization, preferred traits, as well as their yield related traits which still need research attention. The names of the *Amaranthus* genotypes and their origin are given in Table 4.1.

4.2.2 Plant management

In a greenhouse on the premises of the Agricultural Research Council, Vegetable and Ornamental Plants (ARC-VOP), Roodeplaat research farm, Pretoria (25°59’ S and 28°35’ E). Several seeds were sown per cell. Emergence commenced five days after sowing. Thinning was done three weeks after planting to leave one plant per pot.

In the fourth week after planting, the *Amaranthus* seedlings were transported to the Isabelo Co-operative field at KwaMbonambi, KwaZulu-Natal, South Africa (28° 36' 0'' S, 32° 5' 0'' E). Seedlings of each genotype were transplanted to 10 m x 10 m plots with an inter row spacing of 1.5 m and an intra row spacing of 0.3 m. Each genotype had one plot; the experiment was not replicated due to insufficient plant material. Weeding and irrigation was carried out manually when necessary. No fertiliser was applied, since rural farmers typically do not apply fertilizer when cultivating TLVs. Transplanting was done on 14 November 2017 and termination was on 22 March 2018.

4.2.3 Data collection

4.2.3.1 Evaluation of genotypes based on selection criteria

Farmers’ selection was done based primarily on their *Amaranthus* genotypes growing experience, gender ratio and willingness to participate in the research. A total of 14 farmers (ten females and four males) participated in the study. Participants were allowed to set their own selection criteria which were used for evaluation of genotypes. An empty plastic container was then placed in front of each plot. Each participant and a researcher then walked through the plots and discussed the traits of different lines. During that discussion each participant was given a cup filled with bean seeds to select the best genotype for specific traits by dropping between one to four seeds in a container placed in front of the plots. Using a scale from one to four, each participant dropped beans in the containers placed by each plot and this process was repeated for each trait. In ranking exercises the highest score (4) was given for the most preferred traits and the lowest value (1) for the least preferred ones.

For evaluating taste and aroma freshly harvested leaves of each genotype were washed and trimmed before preparation on the day they were evaluated. Harvested leaves were boiled and a pinch of salt was added. A wooden spoon was used to mix the salt until it was completely dissolved, and then cooked *Amaranthus* was kept on separate labelled containers at room temperature and served to evaluate the taste and aroma. Leaf texture was evaluated before and after cooking. In between each sample, panellists rinsed their mouth at least twice with water during a one minute break. A five minute break was given between different plots to prevent fatiguing and also to record each respondent.

Data were collected using score sheets (Appendix 1.2). Questions in the score sheet were asked in vernacular language and responses were filled in by the researcher after listening to the replies and understanding the responses of each participant. The total score was calculated for each criterion. Responses were adapted to a four point Likert Rating Scale (LRS), as poor (P) = 1; good (G) = 2; fair (F) = 3; and excellent (E) = 4. The mean score was computed as 4+3+2+1= 10/4= 2.50. Using the interval score of 0.05 the upper limit cut-off was determined as 2.50 ± 0.05 and the lower limit as 2.55 ± 0.05= 2.45. On the basis of this, mean score (MS) below 2.45 (i.e. < 2.45) were ranked ‘low’; those between 2.45 and 2.54 were considered ‘medium’ (i.e. 2.45 ≥ MS ≤ 2.54); while the mean score greater than or equal to 2.55 (i.e. MS≥ 2.55) were considered ‘high’ (Adeniji and Aloyce 2013).

4.2.3.2 Shelf life determination

For each genotype, forty five leaves from each plot were harvested randomly in the morning and bundled. The bundles were then placed in separate plastic crates and kept in room temperature storage at the co-operative house. *Amaranthus* leaves were then evaluated after seven days on the basis of leaf colour and wilting using the simple 5-rating scales; the limit of acceptance was 2.5 (a score lower than 2.5 indicated poor quality). Colour was determined using a 5-point hedonic scale, where 1= dark-green, 2= light-green, 3= yellowish-green, 4= greenish-yellow, 5= yellow. The extent of wilting was assessed on the basis of a 5-point hedonic scale, where 1 =none, 2 = slight wilting, 3 = moderate wilting, 4 = severe wilting, 5 = extreme.

4.2.4 Data analysis

Descriptive statistics such as frequencies, counts, and percentages were used to analyse the gender and age of the respondents, while Likert Scales was used to analyse the farmer's preferences.

4.3 Results and discussion

4.3.1 Gender and age of the respondents

The number of female respondents across all age categories was higher than the males (Figure 4.1). This observation was possibly because men were mostly at work during the day when PPB was conducted. The ages of respondents ranged from 18 to above 55 years old (Figure 4.1), with the majority being middle aged females between 35 and 55 years old followed by the old age females above 55 years old.

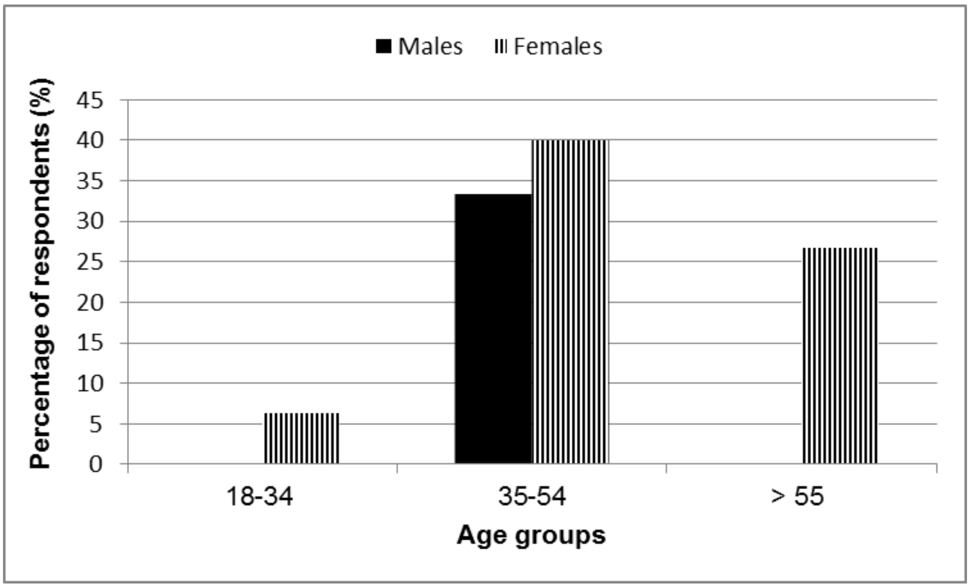


Figure 4.1: Gender and age of the respondents included in participatory genotype selection.

The young age group (18-34 years) was the least numerous of respondents. These results are in accordance with the study of Mofokeng et al. (2014) in the rural areas of Sekhukhune and Waterberg Districts of the Limpopo Province in South Africa where a low level of involvement of the youth in agriculture was reported. The absence of young age males was contrary to the findings of Ntuli (2013) at Umkhanyakude district where most respondents were young-aged males.

4.3.2 Availability and cultivation status of *Amaranthus* genotypes

Of the 15 genotypes tested in this study, only *A.thunbergii* and *A. viridis* were occurring and known in the area, while the rest of the genotypes were unknown to the community prior to this study. According to the respondents, *A. viridis* was occurring abundantly, while *A. thunbergii* was rare because it was believed to be severely affected by drought. In this community, *Amaranthus* was rarely cultivated but harvested on fallowed land and from maize fields, where they are left undisturbed during weeding for future use. Gerrano et al. (2017) and Maseko et al. (2017) also reported that in South Africa, *Amaranthus* is rarely cultivated because as with many other traditional leafy vegetables people believe the plants will grow naturally.

4.3.3 Genotype evaluation according to preferred traits

The traits that were selected by the respondents were stem branching, leaf size, number of leaves per plant, leaf texture, taste and aroma.

4.3.4 Stem branching, leaf size and leaf number

In the current study, genotypes ACAT seed fair, TOT 8789 and AMES-22680 were most preferred for high stem branching (Table 4.2). Stem branching is a desirable horticultural trait that serves as an important index of leaf yield (Gerrano et al. 2017; Munene et al. 2018).

Table 4.2: Respondents' evaluation and ranking of *Amaranthus* genotypes according to stem branching (n= 14)

Amaranthus genotypes	**Scientific name**	**Excellent (4)***	**Good (3)***	**Fair (2)***	**Poor (1)***	**Mean score**	**Rank**
Stem branching							
A. thunbergii	*A. thunbergii*	0 (00)	0 (00)	0 (00)	14 (1)	1	
ACAT seed fair-	*Amaranthus sp.*	6 (1.71)	8 (1.71)	0 (00)	0 (00)	3.42	1
AM-fune	*A. cruentus*	0 (00)	3 (0.64)	11 (1.57)	0 (00)	2.21	
AMES-22680	*A. cruentus*	1 (0.28)	8 (1.71)	5 (0.71)	0 (00)	2.7	2
Anna	*A. cruentus*	0 (00)	0 (00)	0 (00)	14 (1)	1	
Arusha	*A. cruentus*	0 (00)	0 (00)	10 (1.42)	14 (1)	2.42	4
Kobie	*A. cruentus*	0 (00)	0 (00)	0 (00)	14 (1)	1	
TOT 2266	*Amaranthus sp.*	0 (00)	0 (00)	0 (00)	14 (1)	1	
TOT 2358	*Amaranthus sp.*	0 (00)	0 (00)	0 (00)	14 (1)	1	
TOT 4151	*Amaranthus sp.*	0 (00)	0 (00)	10 (1.42)	4 (0.28)	1.7	
TOT 8789	*Amaranthus sp.*	0 (00)	8 (1.71)	6 (0.85)	0 (00)	2.56	3
Tricolor p462179	*A. tricolor*	0 (00)	0 (00)	0 (00)	14 (1)	1	
Tanzania	*Amaranthus sp.*	0 (00)	0 (00)	0 (00)	14 (1)	1	
Thoyandou	*A. greazicans*	0 (00)	0 (00)	0 (00)	14 (1)	1	
W6927N	*A. viridus*	9 (0.64)	5 (1.07)	0 (00)	0 (00)	1.71	

* Number outside bracket represents sample number of respondents (n). Number inside the bracket = sample number of respondents (n) / [the total number of respondents (N) x Likert scale rating].

The highest mean score for bigger leaf size was given to genotypes TOT 8789, ACAT seed fair, AMES-22680, Arusha and W6927N, whereas the lowest mean score was associated with *A. thunbergii* and genotype Tanzania. In a study by Onyango et al. (2008) in Kenya, there was a definite preference for *A. hybridus* compared to *A.cruentus* due to its broad leaves. Mavengahama (2013) concluded that the smaller leaves of *A. greazicans* were identified as a clear drawback during its harvesting. This suggests that bigger leaf size would be a good selection criterion for yield improvement in *Amaranthus* genotypes.

According to the respondents, *A. thunbergii* produces smaller leaves, but a large number of them when subjected to water stress and the opposite when water supply is adequate. This was also evidenced in the current study by *A. thunbergii* ranking last for leaf size but first for number of leaves (Table 4.3). A study by Adeniji et al. (2018) reported high preference for *Amaranthus* genotypes with a large number of leaves per plant. They concluded therefore that from a plant breeder's point of view, such genotypes could serve as donor parents where a large leaf number is sought.

Table 4.3: Respondents’ evaluation and ranking of *Amaranthus* genotypes according to leaf size and number (n=14)

Amaranthus genotypes	**Scientific name**	**Excellent (4)***	**Good (3)***	**Fair (2)***	**Poor (1)***	**Mean score**	**Rank**
Leaf size							
A. thunbergii	*A. thunbergii*	0 (0)	0 (0)	0 (0)	14 (1)	1	
ACAT seed fair-	*Amaranthus sp.*	12 (3.42)	2 (0.14)	0 (0)	0 (0)	3.56	2
AM-fune	*A. cruentus*	0 (0)	0 (0)	10 (1.42)	4 (0.28)	1.7	
AMES-22680	*A. cruentus*	7 (2)	5 (1.07)	2 (0.42)	0 (0)	3.49	3
Anna	*A. cruentus*	0 (0)	8 (1.71)	6 (0.85)	0 (0)	2.56	6
Arusha	*A. cruentus*	4 (1.14)	5 (1.07)	5 (0.71)	0 (0)	2.92	5
Kobie	*A. cruentus*	0 (0)	0 (0)	9 (1.28)	5 (0.35)	1.63	
TOT 2266	*Amaranthus sp.*	0 (0)	0 (0)	5 (0.71)	9 (0.64)	1.35	
TOT 2358	*Amaranthus sp.*	0 (0)	0 (0)	12 (1.71)	2 (0.14)	1.85	
TOT 4151	*Amaranthus sp.*	2 (0.57)	5 (1.07)	4 (0.57)	3 (0.21)	2.42	
TOT 8789	*Amaranthus sp.*	9 (2.57)	5 (1.07)	0 (0)	0 (0)	3.64	1
Tricolor p462179	*A. tricolor*	0 (0)	0 (0)	7 (1)	7 (0.5)	1.5	
Tanzania	*Amaranthus sp.*	0 (0)	0 (0)	11 (1.57)	3 (0.21)	1.78	
Thoyandou	*A. greazicans*	0 (0)	0 (0)	12 (1.71)	2 (0.14)	1.85	
W6927N	*A. viridus*	0 (0)	14 (3)	0 (0)	0 (0)	3	4
Leaf number							
A. thunbergii	*A. thunbergii*	12 (3.42)	2 (0.42)	0 (0)	0 (0)	3.84	1
ACAT seed fair-	*Amaranthus sp.*	5 (1.42)	9 (1.92)	0 (0)	0 (0)	3.34	2
AM-fune	*A. cruentus*	0 (0)	0 (0)	10 (1.42)	4 (0.28)	1.7	
AMES-22680	*A. cruentus*	3 (0.85)	11 (2.35)	0 (0)	0 (0)	3.2	4
Anna	*A. cruentus*	10 (2.85)	4 (0.85)	0 (0)	0 (0)	3.7	3
Arusha	*A. cruentus*	3 (0.85)	11 (2.35)	0 (0)	0 (0)	3.2	4
Kobie	*A. cruentus*	0 (0)	0 (0)	7 (1)	7 (0.5)	1.5	
TOT 2266	*Amaranthus sp.*	0 (0)	0 (0)	4 (0.57)	10 (0.71)	1.28	
TOT 2358	*Amaranthus sp.*	0 (0)	0 (0)	11 (1.57)	3 (0.21)	1.78	
TOT 4151	*Amaranthus sp.*	0 (0)	9 (1.92)	0 (0)	0 (0)	1.92	
TOT 8789	*Amaranthus sp.*	0 (0)	7 (1.5)	0 (0)	0 (0)	1.5	
Tricolor p462179	*A. tricolor*	0 (0)	3 (0.64)	0 (0)	0 (0)	0.64	
Tanzania	*Amaranthus sp.*	0 (0)	0 (0)	8 (1.14)	6 (0.42)	1.56	
Thoyandou	*A. greazicans*	0 (0)	0 (0)	11 (1.57)	3 (0.21)	1.78	
W6927N	*A. viridus*	5 (1.42)	9 (1.92)	0 (0)	0 (0)	3.34	2

* Number outside bracket represents sample number of respondents (n). Number inside the bracket = sample number of respondents (n) / [the total number of respondents (N) x Likert scale rating].

4.3.5 Leaf texture, taste and aroma

Based on both tactile and oral evaluation of the various genotypes, respondents indicated that genotype TOT 8789, followed by Tricolor P462179 were highly preferred for soft texture before and after cooking (Table 4.4). However, for genotype TOT 8789, this softness resulted in mucilaginous texture after cooking which was also undesirable to respondents.

Table 4.4: Respondents' evaluation and ranking of *Amaranthus* genotypes according to leaf texture before and after cooking n= 14

Amaranthus genotypes	**Scientific name**	**Excellent (4)***	**Good (3)***	**Fair (2)***	**Poor (1)***	**Mean score**	**Rank**
A. thunbergii	*A. thunbergii*	3 (0.85)	9 (1.92)	2 (0.28)	0 (0)	3.05	5
ACAT seed fair-	*Amaranthus sp.*	0 (0)	0 (0)	2 (0.28)	12 (0.85)	1.13	
AM-fune	*A. cruentus*	0 (0)	4 (0.85)	7 (1)	3 (0.21)	2.06	
AMES-22680	*A. cruentus*	1 (0.28)	13 (2.78)	0 (0)	0 (0)	3.06	4
Anna	*A. cruentus*	0 (0)	6 (1.28)	8 (1.14)	0 (0)	2.42	
Arusha	*A. cruentus*	4 (1.14)	10 (2.14)	0 (0)	0 (0)	3.28	3
Kobie	*A. cruentus*	0 (0)	1 (0.21)	12 (1.71)	1 (0.07)	1.99	
TOT 2266	*Amaranthus sp.*	0 (0)	4 (0.85)	10 (1.42)	0 (0)	2.27	
TOT 2358	*Amaranthus sp.*	0 (0)	3 (0.64)	11 (1.57)	0 (0)	2.21	
TOT 4151	*Amaranthus sp.*	0 (0)	0 (0)	1 (0.14)	13 (0.92)	1.06	
TOT 8789	*Amaranthus sp.*	14 (4)	0 (0)	0 (0)	0 (0)	4	1
Tricolor p462179	*A. tricolor*	12 (3.42)	2 (0.42)	0 (0)	0 (0)	3.84	2
Tanzania	*Amaranthus sp.*	0 (0)	0 (0)	2 (0.28)	12 (0.85)	1.13	
Thoyandou	*A. greazicans*	0 (0)	0 (0)	0 (0)	14 (1)	1	
W6927N	*A. viridus*	0	7 (1.5)	7 (1)	0	2.5	

* Number outside bracket represents sample number of respondents (n). Number inside the bracket = sample number of respondents (n) / [the total number of respondents (N) x Likert scale rating].

For the purpose of this study aroma was evaluated along with taste on cooked *Amaranthus* genotypes. The genotype ACAT Seed Fair had the highest preference score and ranked first among the respondents with a mean score of 3.92 followed by Tanzania (3.5) and *A. thunbergii*, AMES-22680, Arusha, TOT 4151 and W6927N with a mean score value above 2.45 (Table 4.5). This implied the possibility that any of these genotypes could be selected when breeding for taste. The findings from this study contradict the results obtained by Hiscock et al. (2018), where genotype TOT 2266 obtained the second highest rank for taste while in the current study it was ranked as the eighth most preferred genotype for the same trait (Table 4.5). Similarly, AMES 22680 which was ranked as the fourth most preferred for mild-taste in the current study, was found less acceptable due to bitter taste by Hiscock et al. (2018). Such differences with respect to the taste of two *Amaranthus* genotypes could be attributed by different factors such as storage temperature after harvest, variations in soil type during production (Bett-Garber et al. 2005), fertilisation and geographical factors (Fukuda et al. 2016). However, little is known regarding the relationship between environmental factors, as well as effect of fertilizer amendments on the crop sensory properties (Fukuda et al. 2016).

In addition to favourable taste, genotypes ACAT Seed Fair and Tanzania were also preferred for good aroma (Table 4.5). Genotypes AM-fune (*A. cruentus*) and Thoyandou (*A. greazicans*) were least preferred due to their bitter taste, while Tricolor PI462129 (*A. tricolor*) was described as tasteless. These findings are in consonance with those of Hiscock et al. (2018), at Free state, where respondents also described genotype Thohoyandou and Tricolor PI462129 as bitter taste and tasteless, respectively.

The preference for taste was not affected by gender, with both males and females preferring mild tasting genotypes. These findings are in agreement with Ntuli (2013) who reported that in northern KwaZulu-Natal no gender differences are noted with *Amaranthus* in terms of preferences for taste. It is however contrary to Vorster et al. (2007) and Njume et al. (2014) who reported that men prefer the bitter tasting ones while women and children prefer mild-tasting ones. This lack of gender differentiation in genotype preferences shows that for *Amaranthus* improvement in KwaMbonambi, developing a single genotype that meets the preferences of both males and females is possible.

Table 4.5: Respondents’ evaluation and ranking of *Amaranthus* genotypes according to organoleptic properties n= 14

Amaranthus genotypes	**Scientific name**	**Excellent (4)***	**Good (3)***	**Fair (2)***	**Poor (1)***	**Mean score**	**Rank**
Taste							
A. thunbergii	*A. thunbergii*	7 (2)	7 (1.5)	0 (0)	0 (0)	3.5	3
ACAT seed fair-	*Amaranthus sp.*	13 (3.71)	1 (0.21)	0 (0)	0 (0)	3.92	1
AM-fune	*A. cruentus*	0 (0)	0 (0)	0 (0)	14 (1)	1	
AMES-22680	*A. cruentus*	1 (0.28)	12 (2.57)	1 (0.14)	0 (0)	2.99	4
Anna	*A. cruentus*	0 (0)	0 (0)	0 (0)	14 (1)	1	
Arusha	*A. cruentus*	2 (0.57)	1 (0.21)	11 (1.57)	0 (0)	2.35	
Kobie	*A. cruentus*	0 (0)	0 (0)	3 (0.42)	11 (0.78)	1.2	
TOT 2266	*Amaranthus sp.*	2 (0.57)	3 (0.64)	2 (0.28)	7 (0.5)	1.99	
TOT 2358	*Amaranthus sp.*	0 (0)	0 (0)	0 (0)	14 (1)	1	
TOT 4151	*Amaranthus sp.*	0 (0)	7 (1.5)	7 (1)	0 (0)	2.5	6
TOT 8789	*Amaranthus sp.*	0 (0)	0 (0)	0 (0)	14 (1)	1	
Tricolor p462179	*A. tricolor*	0 (0)	0 (0)	0 (0)	14 (1)	1	
Tanzania	*Amaranthus sp.*	10 (2.85)	4 (0.85)	0 (0)	0 (0)	3.7	2
Thoyandou	*A. greazicans*	0 (0)	4 (0.57)	10 (1.42)	0 (0)	1.99	
W6927N	*A. viridus*	0 (0)	7 (1.5)	7 (1)	0 (0)	2.5	6
Aroma							
A. thunbergii	*A. thunbergii*	7 (2)	7 (1.5)	0 (0)	0 (0)	3.5	2
ACAT seed fair-	*Amaranthus sp.*	13 (3.71)	1 (0.21)	0 (0)	0 (0)	3.92	1
AM-fune	*A. cruentus*	0 (0)	0 (0)	0 (0)	14 (0.21)	0.21	
AMES-22680	*A. cruentus*	1 (0.28)	12 (2.57)	1 (0.14)	0 (0)	2.99	4
Anna	*A. cruentus*	0 (0)	0 (0)	0 (0)	14 (0.21)	0.21	
Arusha	*A. cruentus*	2 (0.57)	1 (0.21)	11 (1.57)	0 (0)	2.35	
Kobie	*A. cruentus*	0 (0)	0 (0)	3 (0.42)	11 (0.78)	1.2	
TOT 2266	*Amaranthus sp.*	2 (0.57)	3 (0.64)	2 (0.43)	7 (0.5)	2.14	
TOT 2358	*Amaranthus sp.*	0 (0)	0 (0)	0 (0)	14 (0.21)	0.21	
TOT 4151	*Amaranthus sp.*	0 (0)	7 (1.5)	7 (1.5)	0 (0)	3	
TOT 8789	*Amaranthus sp.*	0 (0)	0 (0)	0 (0)	14 (0.21)	0.21	
Tricolor p462179	*A. tricolor*	0 (0)	0 (0)	0 (0)	14 (0.21)	0.21	
Tanzania	*Amaranthus sp.*	7 (2)	7 (1.5)	0 (0)	0 (0)	3.5	2
Thoyandou	*A. greazicans*	0 (0)	4 (0.85)	10 (2.14)	0 (0)	2.88	5
W6927N	*A. viridus*	0 (0)	7 (1.5)	7(1.5)	0 (0)	3	3

* Number outside bracket represents sample number of respondents (n). Number inside the bracket = sample number of respondents (n) / [the total number of respondents (N) x Likert scale rating].

4.3.6 Shelf life

Seven days after harvesting, *Amaranthus* leaves changed from green to yellowish green (Table 4.6). The decrease in green colour increased with an increase in storage duration. Assessment further showed that the majority of *Amaranthus* genotypes were given a below average mean score of less than 2.45 for wilting, which implies that respondents viewed all tested genotypes as highly perishable (Table 4.7).

Table 4.6: Respondents evaluation and ranking of *Amaranthus* genotypes according to loss of green colour after seven days duration n= 14

Amaranthus genotypes	**Scientific name**	**dark-green (5)***	**Light green (4)***	**Yellowish green (3)***	**Greenish yellow (2)***	**Yellow (1) ***	**Mean score**	**Rank**
Leaf colour								
A. thunbergii	*A. thunbergii*	0 (0)	0 (0)	14 (4)	0 (0)	0 (0)	4	1
ACAT seed fair-	*Amaranthus sp.*	0 (0)	0 (0)	14 (4)	0 (0)	0 (0)	4	1
AM-fune	*A. cruentus*	0 (0)	0 (0)	14 (4)	0 (0)	0 (0)	4	1
AMES-22680	*A. cruentus*	0 (0)	0 (0)	14 (4)	0 (0)	0 (0)	4	1
Anna	*A. cruentus*	0 (0)	0 (0)	14 (4)	0 (0)	0 (0)	4	1
Arusha	*A. cruentus*	0 (0)	0 (0)	14 (4)	0 (0)	0 (0)	4	1
Kobie	*A. cruentus*	0 (0)	0 (0)	14 (4)	0 (0)	0 (0)	4	1
TOT 2266	*Amaranthus sp.*	0 (0)	0 (0)	14 (4)	0 (0)	0 (0)	4	1
TOT 2358	*Amaranthus sp.*	0 (0)	0 (0)	14 (4)	0 (0)	0 (0)	4	1
TOT 4151	*Amaranthus sp.*	0 (0)	0 (0)	14 (4)	0 (0)	0 (0)	4	1
TOT 8789	*Amaranthus sp.*	0 (0)	0 (0)	14 (4)	0 (0)	0 (0)	4	1
Tricolor p462179	*A. tricolor*	0 (0)	0 (0)	14 (4)	0 (0)	0 (0)	4	1
Tanzania	*Amaranthus sp.*	0 (0)	0 (0)	14 (4)	0 (0)	0 (0)	4	1
Thoyandou	*A. greazicans*	0 (0)	0 (0)	14 (4)	0 (0)	0 (0)	4	1
W6927N	*A. viridus*	0 (0)	0 (0)	14 (4)	0 (0)	0 (0)	4	1

* Number outside bracket represents sample number of respondents (n). Number inside the bracket = sample number of respondents (n) / [the total number of respondents (N) x Likert scale rating].

It was also observed that the soft textured *Amaranthus* were more susceptible to wilting compared to other genotypes. According to Mampholo et al. (2016) leaf yellowing acts as a limiting factor for marketing, apart from wilting, and it indicates that the product has reached the end of its shelf life. Several studies on traditional leafy vegetables also identified their short post-harvest shelf life as the main constraint to their consumption (Onyango et al. 2008; Lewu and Mavengahama 2010; Matenge 2011; Maseko et al. 2017).

Table 4.7: Respondents' evaluation and ranking of *Amaranthus* genotypes according to wilting after seven days duration n= 14

***Amaranthus* genotypes**	**Scientific name**	**Extreme (5)**	**Severe (4)**	**Moderate (3)**	**Slight (2)**	**None (1)**	**Mean score**	**Rank**
Leaf wilting								
A. thunbergii	*A. thunbergii*	0 (0)	14 (4)	0 (0)	0 (0)	0 (0)	4	3
ACAT seed fair-	*A. cruentus*	1 (0.35)	13 (3.71)	0 (0)	0 (0)	0 (0)	4.06	2
AM-fune	*A. cruentus*	0 (0)	14 (4)	0 (0)	0 (0)	0 (0)	4	3
AMES-22680	*A. cruentus*	0 (0)	14 (4)	0 (0)	0 (0)	0 (0)	4	3
Anna	*A. cruentus*	0 (0)	14 (4)	0 (0)	0 (0)	0 (0)	4	3
Arusha	*A. thunbergii*	0 (0)	14 (4)	0 (0)	0 (0)	0 (0)	4	3
Kobie	*Amaranthus* sp.	0 (0)	14 (4)	0 (0)	0 (0)	0 (0)	4	3
TOT 2266	*Amaranthus* sp.	0 (0)	14 (4)	0 (0)	0 (0)	0 (0)	4	3
TOT 2358	*Amaranthus* sp.	0 (0)	14 (4)	0 (0)	0 (0)	0 (0)	4	3
TOT 4151	*Amaranthus* sp.	0 (0)	14 (4)	0 (0)	0 (0)	0 (0)	4	3
TOT 8789	*Amaranthus* sp.	14 (5)	0 (0)	0 (0)	0 (0)	0 (0)	5	1
Tricolor p462179	*Amaranthus* sp.	0 (0)	14 (4)	0 (0)	0 (0)	0 (0)	4	3
Tanzania	*A. viridis*	0 (0)	14 (4)	0 (0)	0 (0)	0 (0)	4	3
Thoyandou	*A. tricolor*	14 (5)	0 (0)	0 (0)	0 (0)	0 (0)	5	1
W6927N	*A. greazicans*	0 (0)	14 (4)	0 (0)	0 (0)	0 (0)	4	3

* Number outside bracket represents sample number of respondents (n). Number inside the bracket = sample number of respondents (n) / [the total number of respondents (N) x Likert scale rating].

4.4 Conclusion

Based on the selection criteria formulated by the respondents themselves in this experiment, genotypes that were mild tasting and high yielding were the most preferred. When multiple traits were considered, genotype ACAT seed fair (*Amaranthus* sp.) ranked first for taste, aroma, leaf size and number of branches. This confirms its position as the most preferred genotype among the 15 evaluated amaranth genotypes. The crop improvement that will target mild tasting and high yielding is therefore essential.

CHAPTER 5

Effect of colchicine treatment on the morphological traits of *Corchorus olitorius* species

Abstract

Polyploidy was induced in Corchorus olitorius L., a traditional leafy vegetable, which is also consumed by the rural community at KwaMbonambi. Corchorus seeds were treated with colchicine at four different concentrations (0.025; 0.05; 0.075 and 0.1 g l^{-1}*) for 2, 4, 6, 8 and 10 hours, respectively. For the control treatment seeds were imbibed in water only. After germination, seedlings were transplanted to seedling trays and then to plastic pots and kept under shade cloth at the University of Zululand. There were three replicates for each treatment in a completely randomized design. Several morphological traits were measured and data were subjected to ANOVA, principal component analysis and cluster analysis. Significant (*$p<0.05$*) variation was observed among all traits measured, with all traits decreasing in value as the colchicine concentration and treatment duration increased, with the exception of stem girth. Of all traits measured, only the leaf chlorophyll content of plants treated with 0.025 g* l^{-1} *colchicine for two hours was significantly (*$p<0.05$*) higher than for the control treatment. Colchicine treatment induced sufficient variability among treatments to be useful in future breeding programmes. The treatments were clustered into three groups based on their similar effect on the morphology of the plants.*

5.1 Introduction

Corchorus olitorius L. is an annual herb of the Tiliaceae family (Nyadanu et al. 2017). It is widely distributed in warm regions throughout the world where it grows spontaneously as a weed in the wild and on cultivated land (Mandal and Datta 2014). *Corchorus* plants serve both for food and medicinal purposes (Masarirambi et al. 2012). Tender leaves are widely eaten as leafy vegetables with starchy dishes either fresh or dried and are cooked into a thick viscous soup or added to stews (Ngomuo et al. 2017). *Corchorus olitorius* leaves are rich in proteins, vitamins and essential amino acids (Gbèdolo et al. 2018).

Colchicine is a highly poisonous alkaloid that is extracted from *Colchicum autumnale* and *C. luteum* (Alam et al. 2011; Kharde et al. 2017). Colchicine is applied to growing tips, meristematic cells, seeds, axillary buds in aqueous solution or mixed with lanoline (Kazi 2012; Noori et al. 2017). It is a widely used antimitotic agent for inducing polyploidy in plants (Salma et al. 2018) and it causes more morphological and molecular mutations than other mutagenic substances (Azoush et al. 2014; Noori et al. 2017). The main action of colchicine is to either prevent the formation of spindle fibres or to inhibit their function during chromosome replication. This, in turn, inhibits the movement of the chromosomes during anaphase. When the daughter chromosomes finally divide, they are all included in one cell and the chromosome number is doubled resulting in a polyploid individual (Alam et al. 2011; Kharde et al. 2017; Li et al. 2018).

Successful colchicine treatment results in plants with improved morphological, physiological and yield traits compared with their diploid counterparts (Wu et al. 2015; Manzoor et al. 2018). The optimum combination of colchicine concentration and duration of treatments is the most important factor for a successful induction of mutation in plants (Salma et al. 2018). In *C. olitorius*, colchicine treatment at low concentration resulted in an increase in plant height, number of leaves, leaf area, number of pods per plant, length of pods and number of seeds per pod (Nura et al. 2011). Low colchicine concentration at short incubation time increased seed germination percentage in *Spinacia oleracea* (Roughani et al. 2017), and plumule length in *Lagenaria sphaerica* (Ntuli and Zobolo 2008). However, an increase in treatment duration increased plumule length in *Lagenaria sphaerica* (Ntuli and Zobolo 2008). Such an interaction between the concentration and duration of colchicine treatments also resulted in an increased plant height in *Vigna unguiculata* (Ajayi et al. 2014) and number of branches in *Catharanthus roseus* (Hosseini et al. 2018). Furthermore, low colchicine concentration and shorter treatment duration resulted in an increase in number of leaves in *V. unguiculata* (Ajayi et al. 2014), leaf chlorophyll content, leaf length and width in *C. roseus* (Hosseini et al. 2018) and petiole length in *Platanus acerifolia* (Liu et al. 2007). Regardless of all the previously-mentioned plant improvements, colchicine treatment is also associated with a number of drawbacks. Some of the common disadvantages of colchicine treatment are: reduction in fertility; inhibition of seed production (Sattler et al. 2016); dwarfism (Ntuli 2007), as well the formation of chimeras (Kazi 2012).

The effect of colchicine treatment was first examined on an *Amaranthus* species, which was the most preferred genus in the study area (Table 3.2), but the experiment failed due to poor seed germination. A similar experiment was then conducted on *C. olitorious* species, since *Corchorus* was the third most preferred genus in the study area. In a study by Nura et al. (2011) the effect of varying colchicine concentrations was investigated on *C. olitorius* with the aim of improving its growth and yield. However, refining colchicine induction protocols for *C. olitorius* could optimize the development of polyploid mutants. This, in turn, will provide genetic diversity with a possibility to select desirable traits for plant improvement. The present study therefore investigates the effect of different colchicine concentrations and treatment durations on the growth and yield traits of *Corchorus olitorius* plants*.*

5.2 Materials and methods

5.2.1 Seed germination procedure

Corchorus olitorius seeds were sourced from Agricultural Research Council, Roodeplaat. Seeds were imbibed in distilled water for one hour. The incubation occurred under light conditions at 25^0C. Three replicates of 20 sterilized seeds each were treated with 10 ml colchicine, with concentrations of 0.025; 0.05; 0.075 and 0.1 g l^{-1} for 2; 4; 6; 8 and 10 hours, respectively, in a completely randomized design. For the control, seeds were germinated in distilled water only. After treating the seeds with colchicine they were rinsed three times in distilled water and transferred to dishes with filter paper to which 10 ml of distilled water had been added. The petri dishes were returned to the incubator until measurements were taken.

5.2.2 Pot experiment

The pot experiment was conducted in a greenhouse at the University of Zululand ($28^0$51' S, $31^0$51' E). The experiment was carried out from August 2017 to February 2018. Seeds of *C. olitorius* were treated with colchicine following the same procedure as in section 5.2.1, but using 150 seeds per dish. Germinated seeds were transferred to seedling trays filled with a commercial germination medium (Hygromix®). The seedling trays were kept in a nursery under shade cloth. At four weeks after transplanting seedlings to the trays, three fully developed seedlings of each treatment were transplanted to a separate 15 L plant pot filled with a loam soil from the University of Zululand farm. Three seedlings were planted in each pot and the pots were kept in a nursery.

The fertilizer recommendation of Allemann and Young (2005) was followed for Swiss chard which is also a leafy vegetable. According to this recommendation, 500 kg/ha of 2:3:4(30) fertilizer was applied per pot at planting. In addition, 225 kg/ha of LAN was applied as a top dressing at four and again at eight weeks after planting.

5.2.3 Data collection

At seven days after returning the petri dishes to the incubator, the seed germination percentage (SG) was determined for each dish. A seed was considered germinated if the radicle protrudes through the seed coat. The radicle (RDL) and plumule length (PLL) (cm) of each germinated seed were measured using a ruler.

Data on vegetative traits were measured at 29 days after transplanting. Plant height (PH) (cm) of the main stem from soil to the stem tip was measured using a measuring tape. Stem girth (STG) (cm) was measured with a Vernier Calliper at 5 cm above ground. The number of branches (NB) and leaves per plant (NL) were counted manually. Leaf area (LA) (cm^2) was calculated as the product of leaf length and width, measured on the fifth fully opened leaf from the apex. Leaf length included a distance between the leaf tip and base, while leaf width entailed measuring the broadest portion of the fifth fully opened leaf from the tip. Petiole length (PTL) (cm) was measured as the distance from main stem to leaf base of the fifth fully opened leaf from the apex.

The method to determine leaf chlorophyll content (LCC) was adopted from Limantara et al. (2015). The relative chlorophyll content of the fifth leaf from the tip of each plant was determined by means of CCM-200 chlorophyll meter (Konica Minolta, Japan). The adaxial side of the leaves was always placed toward the emitting window of the instrument and major veins were avoided. Each leaf was marked with up to 5 representative points for measurements, depending on the size of the leaf, and the average value per leaf was calculated. This was intended to overcome the influence of non-uniform distribution of chlorophyll in the leaf, and hence producing more representative data.

At final harvest (80 days after transplanting); the number of pods per plant (NP/P) was counted manually. Pod length (PDL) (cm) was measured from the base of fruit to the tip of the fruit horn, while pod width (PDW) (cm) was determined from the broadest portion of mature pod using a Vernier Calliper. The number of seeds per pod (NS/PD) of five randomly selected pods per plant was counted and the average number of seeds per pod per plant was calculated.

5.2.4 Data analysis

Data collected were statistically analysed using the Generalized Linear Model (GLM) method using SAS 9.4 (2012). Means were compared using Tukey's Honestly Significant Difference (HSD) test at the 5% significance level. Correlation, principal component analysis, scatter plots (biplots) and agglomerative hierarchical clustering among traits were determined using XLSTAT (2018) by Addinsoft, Paris, France.

5.3 Results

5.3.1 Seedling traits

There was a highly significant ($p<0.01$) decrease in seed germination (SG) of *C. olitorius* seeds with an increase in both colchicine concentration and treatment duration (Table 5.1). Germination percentage of untreated seeds was significantly ($p<0.01$) higher (100%) than all colchicine-treated seeds at different concentrations and durations. Among the colchicine-treated seeds, the highest (58.0%) and the lowest (40.67%) SG was recorded for the 0.025 and 0.1 g l^{-1} colchicine treatments, respectively. Also, treatment for two and ten hours' duration recorded the highest (66.67%) and the lowest (29.16%) SG percentages, respectively.

Table 5.1: Effect of colchicine concentration and treatment duration on seed traits of *C. olitorius* at 7 days after imbibition (n=3)

CC (g l^{-1})	**SG (%)**	**RDL (cm)**	**PLL (cm)**
0	100.0^{a}	1.65^{a}	0.40^{a}
0.025	58.0^{b}	0.91^{b}	0.30^{ab}
0.05	52.0^{bc}	0.67^{bc}	0.31^{ab}
0.075	47.33^{c}	0.63^{bc}	0.20^{b}
0.1	40.67^{d}	0.31^{c}	0.16^{b}
Mean	59.6	0.8	0.2
Significance	<.0001	<.0001	0.0004
Hsd value	6.6	0.3	0.3
Duration (hours)			
0	100.0^{a}	1.65^{a}	0.40^{a}
2	66.67^{b}	1.10^{b}	0.34^{ab}
4	60.83^{b}	0.65^{c}	0.32^{ab}
6	50.0^{c}	0.60^{c}	0.28^{ab}
8	40.83^{d}	0.50^{cd}	0.21^{b}
10	29.16^{e}	0.31^{d}	0.14^{b}
Mean	57.9	0.8	0.26
Significance	<.0001	<.0001	0.2692
Hsd value	7.5	0.4	NS

NS- not significant, CC-colchicine concentration, SG- seed germination percentage; RDL-radicle length; PLL-plumule length.

An increase in colchicine concentration and treatment duration resulted in a highly significant ($p<0.01$) reduction in radicle length (RDL) with the longest radicles (1.65 cm) recorded for seeds of the control treatment (Table 5.1). At the lowest concentration (0.025 g l^{-1}), the RDL was 0.91 cm, while at the highest concentration (0.1 g l^{-1}) it was reduced to 0.31 cm. Shorter treatment duration (2 h) resulted in longer radicles (1.10 cm), while longer treatment duration (10 h) resulted in shorter radicles (0.31 cm).

Significantly ($p<0.01$) shorter plumules (PLL) were recorded at the minimum colchicine concentration of 0.075 g l^{-1} and treatment duration of eight hours when compared with the control (Table 5.1). The longest (0.40 cm) and shortest (0.16 cm) plumules were recorded for seeds of the control treatment and highest (0.1 g l^{-1}) colchicine concentration, respectively. A similar trend was observed with regards to treatment duration with an increase in treatment duration resulting in a decrease in PLL; however differences among treatments were not significant. The PLL among duration treatments varied from 0.14 cm to 0.40 cm.

5.3.2 Stem traits

For both plant height (PH) and number of branches (NB), values decreased with an increase in colchicine concentration with the highest values recorded for the control treatment (71.80 cm and 18.43, respectively) (Table 5.2). Differences between the 0 and 0.1 g l^{-1} colchicine treatments were significant ($p<0.05$) for PH. Duration of treatment had a similar effect in that longer treatment durations resulted in lower values for PH and NB with significant ($p<0.05$) differences between the lowest (zero and two hours) and highest (ten hours) duration treatments.

The trend for stem girth (STG) was the opposite of PH and NB with the highest colchicine concentration (0.1 g l^{-1}) corresponding with plants with the widest stems (4.40 cm) and the lowest colchicine concentrations (0.025 and 0.05 g l^{-1}) with the thinnest stems (1.47 and 1.50 cm, respectively) (Table 5.2). From the two hours to ten hours duration treatments there was a gradual decrease in STG from 3.67 to 1.22 cm, but differences among these treatments were not significant.

5.3.3 Leaf traits

For leaf number (NL), the control had the highest value (74.33) and it decreased significantly ($p<0.05$) as the colchicine concentration increased (Table 5.2). The leaf area (LA) and leaf chlorophyll content (LCC) decreased with an increase in colchicine concentration from 0.025 to 0.1 g l^{-1}. For both LA and LCC, treatment with 0.025 g l^{-1} of colchicine had a higher value than for the control treatment, but it was only significant ($p<0.05$) for LCC. The petiole length (PTL) was 0.30 cm for the control and 0.025 g l^{-1} treatments and 0.20 cm for the remaining treatments.

Table 5.2: Effect of colchicine concentration and treatment duration on vegetative traits of *C. olitorius* at 29 DAT (n=3) and number of pods per plant at 55 DAT (n=5)

CC (g l^{-1})	PH (cm)	STG (cm)	NB	NL	PTL (cm)	LA (cm^{2})	LCC	NP/P
0	71.80^{a}	4.11^{ab}	18.43^{a}	74.33^{a}	0.30^{a}	60.16^{a}	27.28^{bc}	20.70^{ab}
0.025	71.66^{a}	1.47^{b}	18.06^{a}	65.30^{b}	0.30^{a}	70.27^{a}	30.13^{a}	22.0^{a}
0.05	$71.\ 14^{a}$	1.50^{b}	17.66^{a}	63.43^{b}	0.20^{ab}	66.48^{a}	29.60^{ab}	18.96^{bc}
0.075	68.70^{ab}	2.70^{ab}	17.33^{a}	58.80^{b}	0.20^{ab}	62.54^{a}	29.22^{ab}	17.13^{cd}
0.1	61.66^{b}	4.40^{a}	16.76^{a}	50.66^{c}	$0.20a^{b}$	62.38^{a}	27.20^{b}	15.53^{d}
Mean	68.45	2.83	17.64	62.50	0.24	64.36	28.68	18.86
Significance	0.0015	0.0042	0.07	<0.0001	0.0013	0.10	0.03	<0.0001
Hsd value	7.60	2.72	NS	9.87	0.15	NS	2.82	2.75
Duration (hours)								
0	71.80^{a}	4.11^{ab}	17.66^{a}	74.33^{a}	0.30^{a}	60.16a	27.28^{bc}	20.70^{ab}
2	74.43^{a}	3.67^{a}	18.87^{a}	68.54^{ab}	0.34^{a}	72.69^{a}	32.44^{a}	22.33^{a}
4	69.76^{ab}	3.52^{a}	18.0^{ab}	63.70^{abc}	0.32^{a}	69.03^{ab}	29.78^{ab}	20.37^{ab}
6	68.67^{ab}	2.11^{a}	17.70^{ab}	57.70^{bcd}	0.28^{ab}	64.39^{ab}	28.72^{bc}	18.62^{b}
8	65.96^{ab}	2.03^{a}	17.04^{ab}	56.75^{cd}	0.21^{ab}	60.99^{ab}	28.66^{bc}	17.62^{b}
10	61.36^{b}	1.22^{a}	16.62^{b}	51.04^{d}	0.14^{b}	57.01^{b}	25.54^{c}	$0.0\ ^{c}$
Mean	68.66	2.77	17.64	62.01	0.24	64.04	28.73	19.92
Significance	0.0007	0.1114	0.0144	0.0002	0.0080	0.0053	<0.0001	<0.0001
Hsd value	8.67	NS	1.98	11.26	0.17	12.95	3.22	3.14

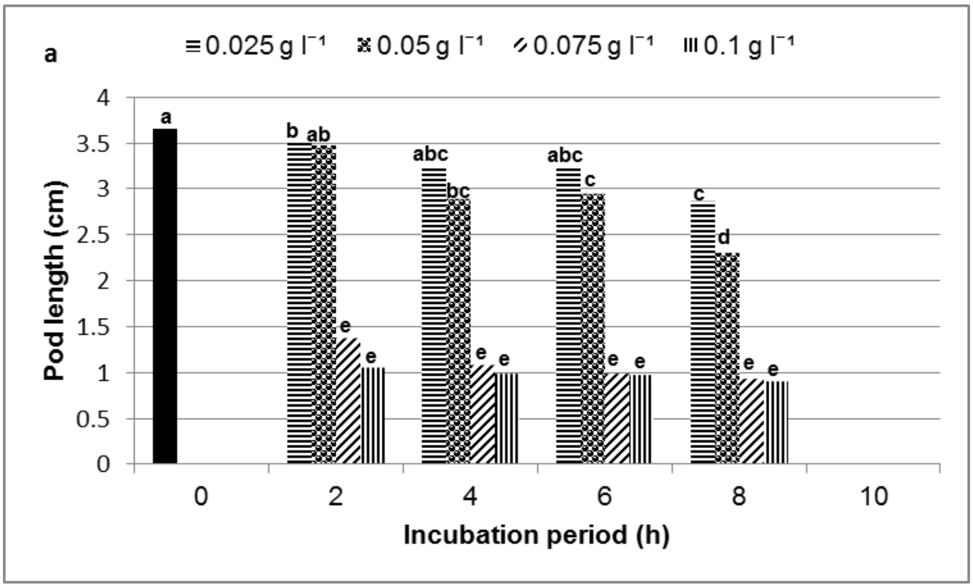
NS-not significant ; CC-colchicine concentration ; PH- plant height; STG-stem girth, NB- Number of branches; NL- number of leaves; PTL- Petiole length; LA- Leaf area; LCC-leaf chlorophyll content; NP/P- number of pods per plant. Means with different letters within a column are significantly different at $p<0.05$ according to Turkeys' HSD.

For all leaf traits, except LA, there was a gradual reduction in values from the two hours to the ten hours duration treatments (significant, $p<0.01$). The control treatment had the highest values for all duration treatments for the control for NL (74.33). For the LA and LCC, however, the values for the 0.025 g l^{-1} treatments were higher than for the control (significant, $p<0.05$ for LCC). The values recorded for these two treatments were 72.69 cm^{2} and 32.44, respectively.

5.3.4 Reproductive traits

Among colchicine-treated plants, the lowest (significant, $p<0.05$) number of pods per plant (NP/P) was recorded at the highest colchicine concentration (0.1 g l^{-1}) and duration (10 h) compared with the control (Table 5.2). The ten hours treatment duration resulted in plants failing to produce pods. The highest NP/P was recorded for the 0.025 g l^{-1} colchicine treatment and where the treatment duration was two hours (22.0 and 22.33, respectively).

A significant ($p<0.05$) interaction between colchicine concentration and treatment duration were observed with regards to pod length (PDL) and pod width (PDW) (Figure 5.1). Among colchicine-treated plants, the longest PDL (3.52 cm) and PDW (0.74 cm) were recorded at 0.025 g l^{-1} of colchicine for 2 h. As the colchicine concentration increased from 0.05 g l^{-1} to 0.075 g l^{-1} there was a drastic and significant ($p<0.05$) decrease in PDL and PDW, irrespective of treatment duration.



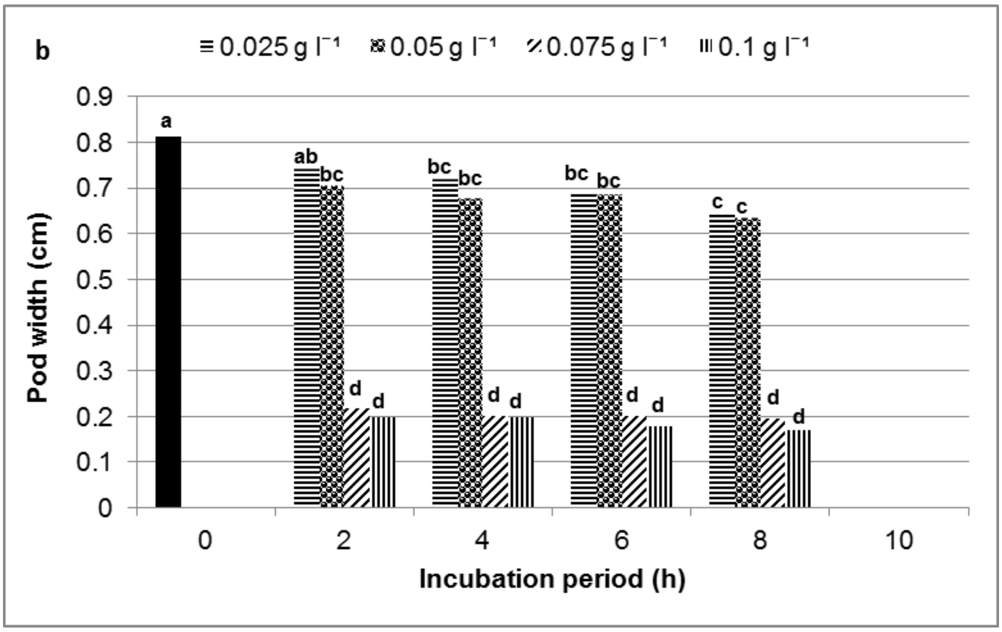


Figure 5.1: Effect of different colchicine concentrations and incubation period on the (a) pod length (PDL) and (b) pod width (PDW). Bars with different letters of the alphabet show significant differences at $p<0.05$.

A significant ($p<0.05$) colchicine concentration x treatment duration interaction was observed in terms of number of seeds per pod (Figure 5.2). Seed production of the control plants was significantly ($p<0.05$) higher (122) than all colchicine-treated plants (Figure 5.2). Any colchicine concentration above 0.05 g l^{-1} resulted in empty pods. Seed production was higher for plants of the 0.025 g l^{-1} colchicine treatment than for the 0.05 g l^{-1} colchicine treatment at all treatment durations, but differences were not significant. There was a decrease in the number of seeds per pod (NS/PD) as the treatment duration increased. Among colchicine treatments, the plants of the 0.025 g l^{-1} colchicine treatment for two hours showed the highest seed production per pod (97.3), while the minimum seed production per pod was recorded at 0.05 g l^{-1} colchicine for eight hours (67.8).

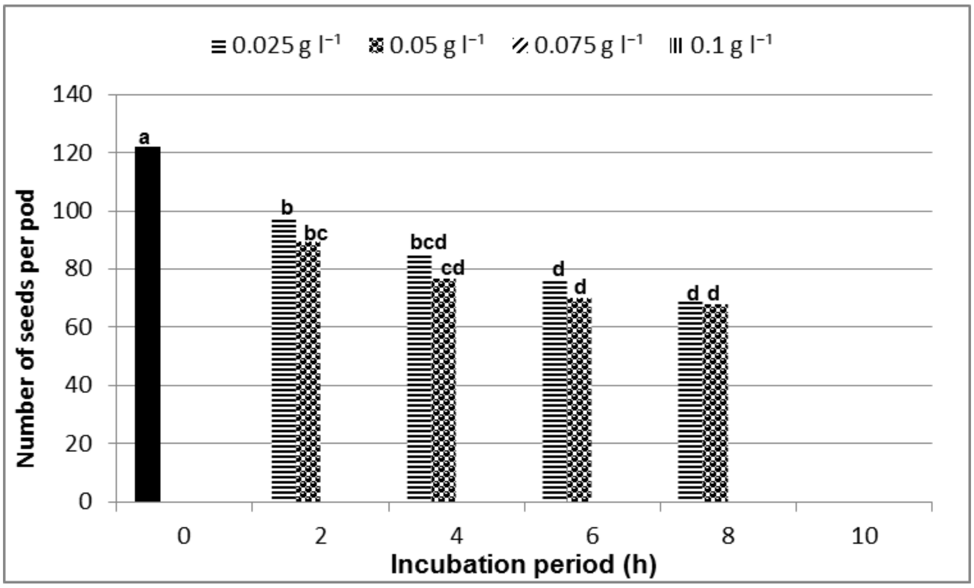


Figure 5.2: Effect of colchicine concentration and treatment duration on the number of seeds per pod (NS/PD) of *C. olitorius.* Bars with different letters of the alphabet show significant differences at $p<0.05$.

5.3.5 Correlation among agronomic traits

All investigated traits in this study had a significant positive correlation with each other except for stem girth (STG) (Table 5.3). Also, leaf chlorophyll content (LCC) had insignificant positive correlations with radicle length (RDL) and number of seeds per pod (NS/PD).

Table 5.3 Pearson correlation coefficients (r^2-values) among traits of *C. olitorius* investigated in this study.

TS	SG	RDL	PLL	NL	LA	LCC	PTL	NB	PH	STG	NP/P	NS/PD	PDL
RDL	**0.762**												
PLL	**0.954**	**0.819**											
NL	**0.868**	**0.808**	**0.934**										
LA	**0.735**	**0.597**	**0.805**	**0.827**									
LCC	**0.634**	0.411	**0.687**	**0.722**	**0.860**								
PTL	**0.581**	**0.570**	**0.704**	**0.752**	**0.867**	**0.719**							
NB	**0.723**	**0.646**	**0.807**	**0.840**	**0.926**	**0.814**	**0.842**						
PH	**0.789**	**0.662**	**0.887**	**0.886**	**0.866**	**0.846**	**0.770**	**0.887**					
STG	0.296	-0.289	0.081	-0.030	0.055	0.182	-0.170	0.002	-0.025				
NP/P	**0.844**	**0.636**	**0.869**	**0.827**	**0.866**	**0.821**	**0.698**	**0.886**	**0.867**	0.216			
NS/PD	**0.741**	**0.841**	**0.787**	**0.792**	**0.635**	0.417	**0.678**	**0.589**	**0.665**	-0.251	**0.634**		
PDL	**0.799**	**0.763**	**0.839**	**0.839**	**0.761**	**0.605**	**0.752**	**0.704**	**0.768**	-0.093	**0.786**	**0.946**	
PDW	**0.754**	**0.757**	**0.807**	**0.805**	**0.715**	**0.540**	**0.725**	**0.659**	**0.735**	-0.158	**0.754**	**0.959**	**0.990**

TS-traits; SG- seed germination percentage; RDL- radicle length; PLL- plumule length; NL- number of leaves; LA- leaf area; LCC- leaf chlorophyll content; PTL- Petiole length; NB- Number of branches; PH- plant height; STG-stem girth, NP/P- number of pods per plant; NS/PD- number of seeds per pod; PDL- pod length; PDW- pod width. Values in bold are significant ($p<0.05$).

5.3.6 Principal component and cluster analyses

Multivariate principal component analysis revealed that the two most informative principal components cumulatively accounted for 84.307% of the total variance (Table 5.4). The first principal component, which explained 72.941% of total variation, was positively defined by all traits except for stem girth. The second component accounted for 11.367% of the total variation and was defined positively by stem girth.

Table 5.4: Principal component (PC) coefficients of traits for different *Corchorus olitorius* plants treated with various colchicine concentrations and treatment duration

Variables	**PC1**	**PC2**
SG	**0.886**	0.158
RDL	**0.805**	-0.394
PLL	**0.949**	0.038
NL	**0.948**	-0.022
LA	**0.909**	0.205
LCC	**0.788**	0.425
PTL	**0.838**	-0.042
NB	**0.897**	0.188
PH	**0.925**	0.128
STG	-0.014	**0.843**
NP/P	**0.912**	0.278
NS/PD	**0.841**	-0.449
PDL	**0.917**	-0.225
PDW	**0.887**	-0.303
Eigenvalue	10.212	1.591
Variability (%)	72.941	11.367
Cumulative (%)	72.941	84.307

PC1-2: Principal components 1-2; SG- seed germination percentage; RDL- radicle length; PLL- plumule length; NL- number of leaves; LA- leaf area; LCC- leaf chlorophyll content; PTL-petiole length; NB-Number of branches; PH-plant height; STG-stem girth; NP/P- number of pods per plant; NS/PD- number of seeds per pod; PDL- pod length; PDW- pod width. Significant ($p<0.05$) values are in bold.

Colchicine treatments were grouped in different quadrants based on their similarity in phenotypic attributes (Figure 5.3). All traits, except stem girth, were positively associated with the first principal component, with the highest variation. These traits were further associated with 0.025 g l^{-1} and 0.05 g l^{-1} colchicine concentrations, both for two and four hour treatment durations. Although colchicine treatment generally retarded growth and yield of *C. olitorius* when compared with the control, these treatments were associated with higher seed germination percentages (SG); longer plumules (PLL); taller plants (PH); higher number of leaves (NL) and pods per plant (NP/P) as well as higher leaf chlorophyll content (LCC), when compared with other treatments.

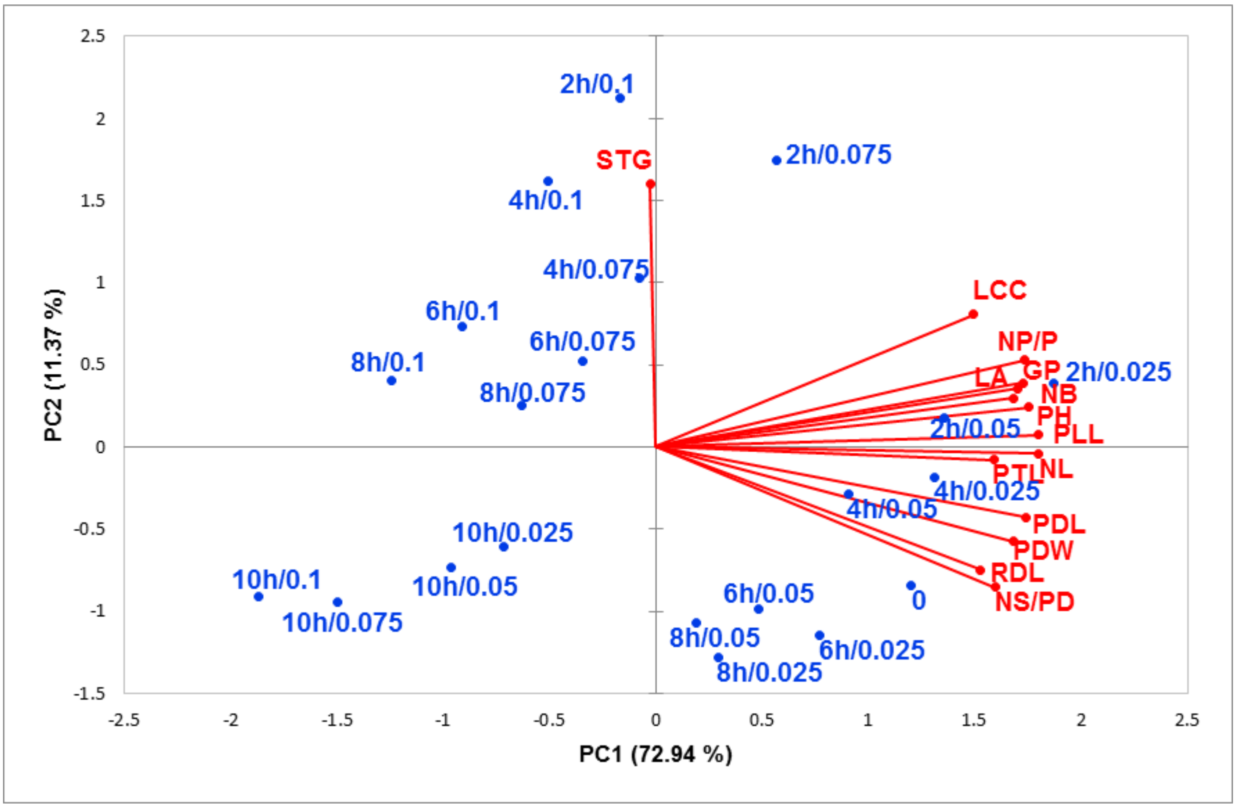


Figure 5.3: Principal component biplot showing variation of colchicine-treated *Corchorus* plants by phenotypic traits.

PC1-2: Principal components 1-2; SG- seed germination percentage; RDL- radicle length; PLL- plumule length; NL-number of leaves; LA- leaf area; LCC- leaf chlorophyll content; PTL-petiole length; NB-Number of branches; PH-plant height; STG-stem girth; NP/P- number of pods per plant; NS/PD- number of seeds per pod; PDL- pod length; PDW-pod width.

The relationship among 21 colchicine treatments was further illustrated by a dendrogram constructed using the unweighted pair group method of arithmetic means (UPGMA) based on Euclidean distance (Figure 5.4). The dendrogram grouped the treatments into two main clusters according to the fourteen evaluated phenotypic traits. Cluster I consisted of three sub-clusters that generally included treatments with either high colchicine concentrations and/or longer treatment durations. Cluster I had the first sub-cluster A which grouped treatments according to high colchicine concentration and higher treatment duration (8h/0.1g l^{-1}; 6h/0.1g l^{-1}; 8h/0.075 g l^{-1}), while subcluster B had similar concentrations but mainly from shorter treatment duration (two and four hours). Sub-cluster C mainly grouped treatments on the basis of low colchicine concentration and higher duration (2h/0.075 g l^{-1}; 10h/0.05 g l^{-1} and 10h/0.05 g l^{-1}).

Cluster II had two sub-clusters with a clear grouping mainly according low colchicine concentration. Subcluster A included treatments mainly from low colchicine concentrations and longer treatment durations, while subcluster B comprised mostly of treatments with low colchicine concentrations and shorter treatment durations (2h/0.05 g l^{-1}; 4h/0.025 g l^{-1} and 2h/0.05 g l^{-1}) assembled with the control.

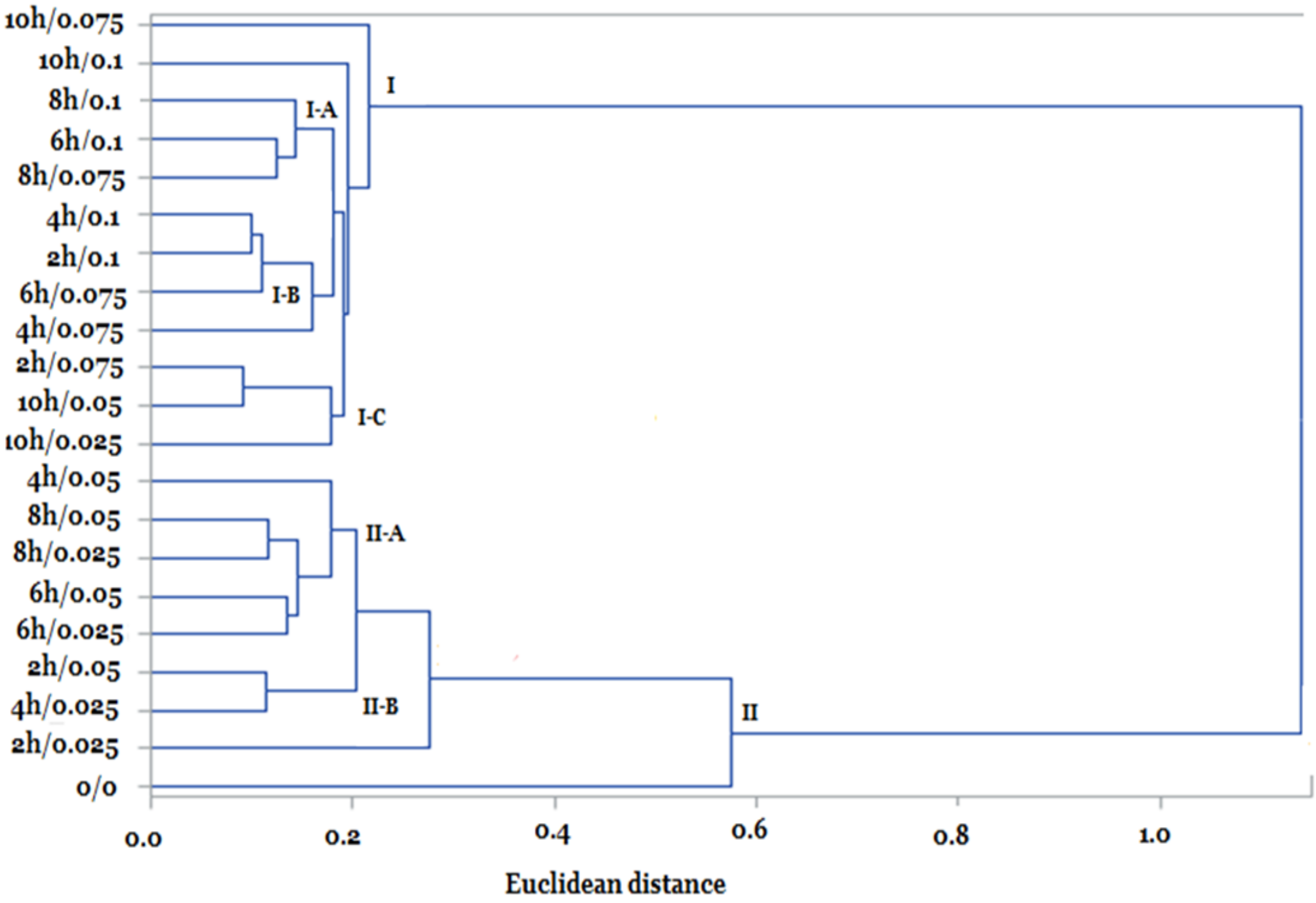


Figure 5.4 Unweighted pair group method of arithmetic means (UPGMA) dendrogram based on Euclidean distance, summarising data on differentiation among the twenty one treatments of this study.

5.4 Discussion

5.4.1 Variation in growth and yield traits

The decrease in seed germination percentage (SG) of *C. olitorius* seeds with an increase in colchicine concentration observed in the current study was similar to findings for *Vigna unguiculata* (Essel et al. 2015), *Praecitrullus fistulosus* (Rafiq et al. 2016), *Spinacia oleracea* (Roughani et al. 2017) and *Taraxacum kok-saghyz* (Luo et al. 2018). Similarly, reductions in SG percentage with increasing colchicine concentration and treatment durations have also been reported in *Sesamum indicum* (Mensah et al. 2007), *Vigna unguiculata* (Essel et al. 2015) and *Agastache foeniculum* (Talebi et al. 2016). These reductions could be a result of colchicine toxicity that prevented treated seeds from emerging (Roughani et al. 2017; Luo et al. 2018).

In each colchicine concentration there was a decrease in radicle length (RDL) as the incubation period increased from 2 to 10 h. Also, an increase in colchicine concentration from 0.025 to 0.1 g l^{-1} reduced RDL in all the treatments, compared to the control (Table 5.1). These results agree with the findings on *Sesamum indicum*, where high colchicine concentration decreased root length (Mensah et al. 2007). Similarly, high colchicine concentrations and treatment durations inhibited radicle elongation in *Taraxacum kok-saghyz* (Luo et al. 2018) and *Lagenaria sphaerica* (Ntuli and Zobolo 2008). Such reductions in RDL of colchicine-treated seeds might be associated with residual colchicine in seed coat, which later inhibits seedling growth by reducing cell division in the longitudinal direction (Hosseini et al. 2018; Luo et al. 2018).

Plumules of the control seeds were longer than those that were treated with colchicine (Table 5.1). There was a reduction in plumule length (PLL) as the concentration and treatment duration increases. These findings concur with the results in *Pogostemon cablin*, where colchicine-treated explants had significantly shorter shoots than untreated explants (Yan et al. 2016). Also, PLL of colchicine treated *C. palmata* and *L. sphaerica* plants decreased with an increase in treatment durations (Ntuli and Zobolo 2008).

Plant height (PH) was significantly reduced by the highest colchicine concentration and the longest treatment duration (Table 5.2). Similar results of such an interaction between the concentration and duration of colchicine treatments was also reported in *Datura stramonium* (Amiri et al. 2010); *Echinacea purpurea* (Abdoli et al. 2013), *Phlox drummondi* (Tiwari and Mishra 2012), *Vigna unguiculata* (Ajayi et al. 2014), *and Gladiolus grandiflorus* (Manzoor et al. 2018). In contrast, high colchicine concentrations resulted in a significant increase in PH of *Solanum tuberosum* (Alam et al. 2011) and *Solanum rebaudiana* (Mahdi et al. 2018). In other studies, colchicine-treated *Lagerstroemia indica* (Ye et al. 2010) and *Catharanthus roseus* (Hosseini et al. 2018) plants showed no change in PH when compared with the control. Such findings support, Hannweg et al. (2016) who mentioned that the direction of morphological change for colchicine-treated plants for a particular species cannot be anticipated.

A reduction in plant height (PH) with an increase in duration of treatment is very common among mutated crops. This is linked to the reduction in the rate of cell division among treated plants, chromosomal abnormality, reduction of auxin levels, inhibition of auxin synthesis and failure of assimilation mechanisms (Ajayi et al. 2014). The toxic effect of colchicine on cell division (Salma et al. 2018) and colonic mutation factor from colchicine might also reduce PH in colchicine-treated plants (Yunus et al. 2018).

Stem girth (STG) of *C. olitorius* increased with an increase in colchicine concentration but decreased with an increase in treatment duration (Table 5.2). Similarly, application of higher doses of colchicine resulted in maximum STG in *Trachyspermum ammi* (Noori et al. (2017). Further, in *Linum album* (Javadian et al. 2017) and *Artemisia annua* (Yunus et al. 2018) prolonged treatment duration irrespective of colchicine concentration, resulted in decreased STG.

There was a gradual decrease in number of branches (NB) per plant with an increase in colchicine concentrations and treatment durations (Table 5.2). On the contrary, in *Datura stramonium* (Amiri et al. 2010) and *Vigna unguiculata* (Essel et al. 2015) the NB increased with an increase in colchicine concentration and incubation times. A reduction in NB in colchicine-treated plants has also been reported in *Vigna unguiculata* (Amiri et al. 2010) and *Psophocarpus tetragonolobus* (Udensi et al. 2017).

In the present study, the number of leaves (NL) decreased with an increase in colchicine concentration (Table 5.2). Similar dose-dependent effects of colchicine on leaf production were reported in *Gossypium arboretum* (Rauf et al. 2006), *Sesame indicum* (Mensah et al. 2007), *Datura stramonium* (Amiri et al. 2010) and *Linum album* (Javadian et al. 2017). However, studies on *Phlox drummondi* (Tiwari and Mishra 2012), *Vigna unguiculata* (Essel et al. 2015) and *Gladiolus grandiflorus* (Manzoor et al. 2018), showed an increase in the NL with an increase in colchicine concentration.

The petiole length (PTL) of *C. olitorius* plants decreased with increasing concentration of colchicine and treatment duration (Table 5.2). The results of the present study agree with reports on *Platanus acerifolia* where the PTL of colchicine-treated plants became shorter with an increase in colchicine concentration (Liu et al. 2007).

Colchicine concentration did not significantly affect leaf area (LA) of *C. olitorius*, but was highest in the 0.025 g l^{-1} colchicine treatment in the current study (Table 5.2). However, studies by Yan et al. (2016) and Manzoor et al. (2018) did show significantly higher LAs at low colchicine concentrations treatments relative to high colchicine concentration treatments. Also, Chen et al. (2009) observed a significant reduction in the LA of colchicine-treated *Paratomoxia hieroglyphica,* in comparison to their respective untreated counterparts. Prolonging the duration of treatment decreased LA in *C.olitorius* in the current study, but not significantly. Hosseini et al. (2018) as well as Zahumenicka et al. (2018) did, however, find a significant reduction in LA with an increase in treatment duration in *Anemone sylvestris* and *Catharanthus roseus*, respectively.

The maximum leaf chlorophyll content (LCC) was associated with low colchicine concentration treatments and LCC was reduced as the concentration increased (Table 5.2). The reduction in LCC due to increased concentration of colchicine also agrees with findings in *Catharanthus roseus* (Hosseini et al. 2018) and *Jatropha curcas* (Manzoor et al. 2018). Contrastingly, the colchicine-treated *Sesame indicum* (Mensah et al. 2007); *Datura stramonium* (Amiri et al. 2010) and *Cannabis sativa* (Bagheri and Mansouri 2015) showed an increase in LCC with an increase in colchicine concentration and treatment duration. The accumulation of high chlorophyll contents in the leaves of colchicine-treated plants may be due to an increased number of chloroplasts in the stomatal guard cells (Udensi et al. 2017; Hosseini et al. 2018). It is also a result of facilitation of the absorbance of minerals that promote the formation of chlorophyll and activation of key enzymes for carbon fixation (Zahumenicka et al. 2018).

The higher values observed for vegetative traits at low colchicine concentrations relative to high colchicine concentrations in the current study might be due to the stimulatory influence of this compound on plant morphogenesis. Contrastingly, colchicine at higher doses lead to C-mitoses that inhibits cell multiplication, leading to reduced vegetative parameters in colchicine-treated plants (Barman et al. 2015).

Low colchicine concentrations and shorter treatment duration decreased the number of pods in *C. olitorius* plants (Table 5.2). The results from the present study further showed that higher colchicine concentration inhibited pod development in all treatment durations. Similar findings were recorded in *C. olitorius* (Nura et al. 2011), *Phalaenopsis amabilis*

(Azmi et al. 2016) and *Vigna unguiculata (*Ajayi et al. 2014), where low colchicine dosages resulted in an increase in the number of pods produced per plant. The reduction in pod number of colchicine-treated plants may be as a result of inhibiting the action of enzymes, changes in enzymatic activities and toxicity of the mutagen that is achieved at the highest concentration (Ajayi et al. 2014; Azmi et al. 2016).

Colchicine significantly reduced the number of seeds per pod (NS/PD) in all treatments compared with the control (Figure 5.2). Among colchicine-treated seeds, the NS/PD decreased with increasing concentration of colchicine and treatment duration. These results agree with the findings of Abdoli et al. (2013) in *Echinacea purpurea* where colchicine-treated plants showed reduction in seed number with an increase in concentration and treatment duration. However, this is contrary to *Phaseolus vulgaris* (Ajayi et al. 2014) and *Psophocarpus tetragonolobus* (Udensi et al. 2017), where higher levels of colchicine and duration led to an increase in the number of seeds produced by each pod.

High colchicine concentration and long treatment duration had a significant inhibiting effect on the pod length (PDL) and width (PDW) of *C. olitorius* plants (Figure 5.1a and 5.1b). These findings are in line with Obute et al. (2007) and Nura et al. (2011), in *Vigna unguiculata* and *Corchorus olitorius* species whose results also revealed that the PDL and PDW decreased with an increase in colchicine concentration. It also agrees with Azmi et al. (2016), where higher colchicine concentrations resulted in shorter pods in *Phalaenopsis amabilis*.

As expected, the longer and bigger pods of untreated plants permitted an increase in the pod surface area that accounted for an increment in the number of seeds produced per pod (NS/PD). Similar findings were recorded by Ajayi et al. (2014) and Azmi et al. (2016) in *Vigna unguiculata* and *Phalaenopsis amabilis*, respectively, where colchicine treatment reduced the number of seeds produced per pod when compared with the control. The reduction in the seed number of colchicine-treated plants may be due to their increased seed size which eventually results in fewer seeds produced per pod than that of untreated ones (Hosseini et al. 2018). However, seed size was not measured in the current study.

5.4.2 Correlations

In the present study, the Pearson’s correlation coefficients were positive and significant ($p<0.05$) for almost all studied traits (Table 5.3). Plant height (PH) showed positive and highly significant correlation with leaf number (NL), leaf area (LA), and number of branches (NB), which agree with reports on *Solanum macrocarpon* (Nyadanu et al. 2014) and *Cicer arietinum* (Tesfamichael et al. 2015). The current study also revealed a significant and positive correlation between NL and LA. Similar results were reported in *Cleome gynandra* where NL correlated positively with the LA (Wasonga 2014). In *Cicer arietinum*, the NL per plant also correlated positively with PH and NB per plant (Tesfamichael et al. 2015) which corresponds with the current study.

In *C. olitorius*, the number of pods per plant (NP/P) correlated positively with plant height (PH), leaf number (NL), leaf area (LA), pod length (PDL), pod width (PDW) and number of seeds per pod (NS/PD) in the current study. Similarly, a positive correlation was observed between NS/PD and PDL of *Vigna unguiculata* (Okoronkwo and Nwofia 2016). The significant ($p<0.05$) and positive correlation that existed among the majority of the morphological traits in colchicine treated *C. olitorius* provides an opportunity to improve desirable morphological traits simultaneously.

5.4.3 Principal component and cluster analyses

The results of principal component analysis showed that the first two principal components (PC1 and PC2) contributed more than 80% of the total variation (Table 5.4). Leaf number (NL), leaf area (LA), plant height (PH), NP/P and PDL were indicated by principal component analysis as the most reliable morphological traits that contributed to total variation. The presence of such variation was of importance because an integral factor in breeding and production of leafy vegetables is increment of the leaf size (Roughani et al. 2017). The diversity in morphological traits among colchicine-treated plants implies that the various colchicine concentrations and treatment durations evaluated in the current study successfully increased genetic variability in *C. olitorius* plants. This further indicated that potential traits for breeding programmes could be obtained from treatments in first and second principal components.

The dendrogram clustered the colchicine treated *Corchorus* plants into two major clusters based on the eleven phenotypic traits evaluated (Figure 5.4). Cluster I consisted of three sub-clusters that grouped treatments mainly according to high colchicine concentration. These *C. olitorius* plants were associated with reduction in all traits studied, except stem girth. The cluster analysis further showed that cluster II of the dendrogram was the most appropriate group for varietal improvement. It had several features of interest namely, high seed germination percentage, taller plants with numerous leaves, higher total chlorophyll content, highest number of pods per plant as well as longer pod length and width relative to the other treatments. All of these traits decreased with an increase in both colchicine concentration and treatment duration.

Clustering showed a close relationship between the *C. olitorius* plants treated with low colchicine concentrations and shorter treatment durations. Likewise, those treated with high colchicine concentrations and longer treatment durations were mainly clustered together. According to Nyadanu et al. (2014) and Odour (2016), accessions in a cluster are more genetically similar than the counterparts in other cluster groups while those that are grouped in different clusters are genetically different and could be used as parent lines for the genetic improvement (Gerrano et al. 2015).

The high level of similarity among plants from low colchicine concentration and shorter treatment duration and those from high concentration and longer duration was also observed in the biplot. Low colchicine concentration (0.025 g l^{-1} and 0.05 g l^{-1}); shorter treatment duration (two and four hours) as well as the control were clustered together in positive association with the first principal component, in relation to all traits except stem girth. However, the remaining higher concentrations and longer treatment durations were isolated from other treatments and were considered to be more diverse but inferior in terms of all morphological traits, excluding stem girth. Such findings are in accordance with Kazi (2012), who reported that colchicine treatment was most efficient at very low concentration and duration. The presence of wide phenotypic variation as a result of colchicine-treatment showed the potential of this chemical for improving and developing high yielding *C. olitorius* plants. High leaf yield is one of the main criteria for identifying and selecting superior varieties for most farmers. Thus, promising and high yielding plants from this study could be used directly by the farmers or exploited for future hybridization of *C. olitorius* species (Tesfamichael et al. 2015). These factors could also be used as useful marker traits that most effectively discriminate between colchicine-treated *C. olitorius* accessions (Nyadanu et al. 2014).

5.5 Conclusion

There was a decrease in all measured traits with an increase in colchicine concentration and treatment duration except for stem girth which increased with an increase in colchicine concentration. The colchicine treatment with 0.025 g l^{-1} and 0.05 g l^{-1} of colchicine for two and four hours showed higher seed germination percentage (GP); longer plumules (PLL); taller plants (PH); higher number of leaves (NL) and pods per plant (NP/P) when compared with other treatments, but not the control treatment. Only the leaf chlorophyll content (LCC) of the 0.025 g l^{-1} treatment and two hour duration treatment were significantly higher than the control. The level of dissimilarity among the plants as a result of treatment was also high and this indicates an effectiveness of colchicine treatment on the creation of genetic variability.

CHAPTER 6

Variability of the leaf mineral content in colchicine-treated *Corchorus olitorius*

Abstract

The effect of colchicine concentration and treatment duration on the leaf mineral content of Corchorus olitorious, a popular traditional leafy vegetable (TLV), was investigated. Corchorus olitorious seeds were treated with 0.025; 0.05; 0.075 and 0.1 g l^{-1} *of colchicine for 2, 4, 6, 8, and 10 hours, respectively, in a completely randomized design with 3 replicates. For the control treatment seeds were imbibed in distilled water only for an hour. After treatment with colchicine, seeds were rinsed in sterile distilled water and sown in a commercial growth medium (Hygromix®) in polystyrene trays and later transplanted to potted soil. Three seedlings were planted per pot and kept under a shade cloth at the University of Zululand. Samples of young and tender leaves were taken at their vegetative stage for chemical analysis. The leaves were analysed for their nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), aluminium (Al), and zinc (Zn), manganese (Mn), copper (Cu) and iron (Fe) using atomic absorption spectrophotometry. Data were subjected to ANOVA, principal component analysis and cluster analysis. Treatment with 0.025 g* l^{-1} *of colchicine for two hours significantly (*$p<0.05$*) increased the N, P, K, Ca, Mg, Al and Fe content in the leaves of C. olitorious plants when compared with the untreated plants. The study demonstrated high levels of variation among the colchicine treatments in the nutritional traits evaluated. This implies that colchicine has the potential of being used for future C. olitorious crop improvement.*

6.1 Introduction

Traditional leafy vegetables are important sources of macro and micronutrients which are essential for human health (Ntuli 2013). Plants classified as TLVs are widely consumed and can serve as an important source of macro and micronutrients, essential minerals, carbohydrates, proteins, vitamins and certain hormone precursors (Naim et al. 2015). Most TLVs are comparable or have higher nutrient content than their fully domesticated counterparts (Legwaila et al. 2011).

South Africa has a large biodiversity of TLVs including *Corchorus olitorius* which has a remarkably high nutrient content. *Corchorus olitorius* is an annual herb that belongs to the Family Tiliaceae (Ndlovu and Afolayan 2008). It is widely consumed as a leafy vegetable among rural communities in most parts of Africa (Masarirambi et al. 2012). The young leaves are cooked as vegetables and immature fruits are added to salads (Naim et al. 2015). Its leaves contain large amounts of phenols, amino acids and carotenoids (Giro and Ferrante 2017). It is also a very good source of proteins, lipid, carbohydrates, vitamins (A, C, E) and mineral nutrients like calcium, iron (Nyadanu et al. 2017), potassium and magnesium (Njoumi et al. 2018). Despite its high nutritional content, the nutritive value and micronutrient bioavailability of *C. olitorius* has not been well researched in South Africa (Ndlovu and Afolayan 2008).

Being easily accessible to the underprivileged households, especially in rural areas, *C. olitorious* consumption may contribute to solving the problem of nutritional deficiencies among these populations (Lewu and Mavengahama 2010). Therefore, further improvement in their nutritional quality is a major goal of the plant breeding sector (Tabbita et al. 2017). Genetic manipulation with colchicine is one of the most commonly used methods to develop nutritionally improved crop varieties (Chakraborty and Paul 2013). The mutagen colchicine is a poisonous compound extracted from seeds and bulbs of *Colchicum autumnale* and is mainly applied to seeds in its liquid form (Kazi 2012). Colchicine treatment often produces immediate changes in the structural characteristics, biochemistry, and physiology of the plants, which often influence their nutritional content, in addition to other effects (Ghimire et al. 2016). The effectiveness of this mutagen for a particular plant species depends on the dose and incubation time because of differences in plant species' responses towards colchicine application (El-Morsy et al. 2009).

Colchicine treatment increased the amount of calcium, magnesium and phosphorus in *Nicotiana alata* leaves (El-Morsy et al. 2009). So far, few studies have been done on the effects of colchicine treatment on the leaf mineral elements of consumable plants (Bagheri and Mansouri 2015). Also, to the best of the researcher's knowledge, there are no or few reports on the effects of colchicine concentration and treatment duration combined, on the leaf mineral content of *C.olitorius*. The present study therefore aimed to determine the effect of colchicine concentration and treatment duration on leaf mineral content of *C. olitorius* plants.

6.2 Materials and Methods

6.2.1 Sample preparation and determination of leaf mineral content

The *C. olitorius* leaves were collected once off during their vegetative stage from the same trial as reported in Chapter 5. Young and tender leaves of the same maturity were collected from each plant. Leaves were washed with distilled water and oven dried at 60˚C (Labcon incubator, Model LTIM). They were then ground into a fine powder using a laboratory grinder (Ultra centrifugal Mill ZM 200), sieved and stored in air-tight containers under room temperature prior to analysis. Samples were sent to the Soil Fertility and Analytical Services section of the Department of Agriculture and Rural Development of KwaZulu-Natal province and analysed for their nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), aluminium (Al), zinc (Zn), manganese (Mn), copper (Cu) and iron (Fe) content according to Manson and Roberts (2000). The K, Ca, Mg, and Na, Zn, Mn, Cu content were determined by atomic absorption. Phosphorus content was determined by the molybdenum-blue procedure according to Murphy and Riley (1958). Total nitrogen (N) was determined from a wet acid digest of sample by colorimetric analysis (Eckard 1988).

6.2.2 Data analysis

Data collected were statistically analysed using the Generalized Linear Model (GLM) method using SAS 9.4 (2012). Means of leaf minerals were compared using Tukey’s Honestly Significant Difference (HSD) test at the 5% significance level. Multivariate analysis was performed to identify the most significant descriptors in capturing the nutrient variation using XLSTAT (2018). Nutritional traits were subjected to correlation, principal component analysis, and biplots to discriminate and group different treatments, respectively.

6.3 Results

6.3.1 Leaf mineral content

The N, P, K, Ca, Mg, Al and Fe content of C*. olitorious* leaves were significantly ($p<0.01$) higher for the 0.025 g l^{-1} colchicine treatment than for the control treatment (Table 6.1). The same minerals were also significantly ($p<0.05$) higher for leaves of the two hour duration treatment relative to the control. From a colchicine concentration of 0.025 to 0.1 g l^{-1} there was a gradual decrease in the content of these minerals with significant ($p<0.01$) differences recorded between the 0.025 g l^{-1} and 0.1 g l^{-1} colchicine treatments. There was also a decrease in N, P, K, Ca, Mg, Mg, Al and Fe of the leaves as the duration of treatment increased from 2 to 10 h with leaves of the 2 hour duration treatment having significantly ($p<0.01$) higher values than leaves of the 10 hour treatment.

For both the Na and Mn content of leaves, values increased with an increase in colchicine concentration from 0.025 to 0.1 g l^{-1} with the highest values recorded for maximum colchicine concentration of 0.1 g l^{-1} (227.50 mg kg^{-1} and 119.60 mg kg^{-1}, respectively) (Table 6.1). Leaves of plants of the 2 h duration treatment showed the highest Na and Mn content (530.60 mg kg $^{-1}$ and 123.20 mg kg^{-1}, respectively) , for all duration treatments.

The Zn content of leaves of untreated plants was significantly ($p<0.01$) higher (30.33 mg kg^{-1}) than leaves of all colchicine-treated plants and decreased with an increase in both colchicine concentration and treatment duration (Table 6.1). The leaf Zn content of the 0.1 g l^{-1} colchicine treatment and 10 hour duration treatment were 18.90 and 21.50 mg kg^{-1}, respectively.

Colchicine concentration and treatment duration did not have a significant effect on the Cu content of leaves. Values varied from 7.52 (for the 0.025 g l^{-1} colchicine treatment) to 8.80 mg kg-1 (for the 0.1 g l^{-1} colchicine treatment) and from 7.73 mg kg^{-1} to 9.0 mg kg^{-1} for the 10 hour and 2 hour duration treatments, respectively.

Table 6.1: Mineral content of colchicine treated *C.olitorius* plants

CC (g l^{-1})	**Macronutrients (%)**					**Micronutrients (mg kg^{-1})**					
	N	**P**	**K**	**Ca**	**Mg**	**Na**	**Al**	**Zn**	**Mn**	**Cu**	**Fe**
0	2.40^{c}	0.37^{b}	2.53^{bc}	1.20^{c}	0.41^{c}	596.50^{a}	60.00^{d}	30.33^{a}	117.00^{ab}	7.97	91.00^{e}
0.025	2.80^{a}	0.50^{a}	2.80^{a}	1.40^{a}	0.50^{a}	182.90^{b}	123.13^{a}	26.50^{b}	102.46^{c}	7.52	152.20^{a}
0.05	2.60^{b}	0.42^{ab}	2.70^{ab}	1.30^{b}	0.46^{b}	196.90^{b}	105.73^{b}	23.60^{bc}	106.27^{bc}	8.07	131.00^{b}
0.075	2.43^{c}	0.40^{ab}	2.56^{bc}	1.24^{c}	0.44^{b}	207.91^{b}	91.06^{c}	21.60^{cd}	112.60^{abc}	8.47	118.13^{c}
0.1	2.30^{d}	0.37^{b}	2.40^{c}	1.10^{d}	0.30^{c}	227.50^{b}	64.53^{d}	18.90^{d}	119.60^{a}	8.80	104.47^{d}
Mean	2.50	0.40	2.60	1.30	0.40	282.30	88.84	24.20	111.30	8.20	119.40
Significance	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	0.0985	<0.0001
Hsd value	0.10	0.10	0.21	0.12	0.14	61.90	8.23	3.71	13.10	NS*	11.92
Duration (hours)											
0	2.40^{d}	0.37^{b}	2.53^{bc}	1.20^{c}	0.41^{c}	596.53^{a}	60.00^{d}	30.33^{a}	117.00^{ab}	7.97	91.00^{e}
2	2.60^{a}	0.50^{a}	2.70^{a}	1.32^{a}	0.50^{a}	530.60^{ab}	103.30^{a}	24.08^{b}	123.20^{a}	9.00	136.08^{a}
4	2.56^{a}	0.43^{ab}	2.62^{ab}	1.30^{ab}	0.50^{a}	480.74^{b}	98.60^{ab}	23.08^{b}	115.83^{ab}	8.63	130.42^{ab}
6	2.53^{ab}	0.41^{b}	2.60^{ab}	1.25^{abc}	0.46^{a}	380.71^{b}	95.75^{abc}	22.50^{b}	110.70^{abc}	8.30	125.50^{ab}
8	2.47^{bc}	0.40^{bc}	2.60^{ab}	1.24^{c}	0.45^{ab}	330.60^{b}	93.41^{c}	22.00^{b}	103.92^{bc}	7.96	121.83^{b}
10	2.43^{cd}	0.35^{c}	2.50^{b}	1.22^{c}	0.42^{bc}	298.42^{c}	89.50^{c}	21.50^{b}	97.60^{c}	7.73	118.42^{b}
Mean	2.50	0.40	2.60	1.30	0.40	436.27	90.0	23.90	111.40	8.20	120.5
Significance	<0.0001	<0.0001	0.0108	<0.0001	<0.0001	<0.0001	<0.0001	0.0003	0.0001	0.05	<0.0001
Hsd value	0.12	0.11	0.21	0.13	0.03	66.21	8.72	3.91	13.11	NS*	12.72

NS- not significant; CC- colchicine concentration; Hsd- Honest significant difference, N- nitrogen; P- phosphorus; K- potassium; Ca- calcium; Mg- Magnesium; Na- Sodium; Al- aluminium; Zn- Zinc; Mn- Manganese; Cu- Copper; Fe- Iron. Means with different letters within a column are significantly different at $p<0.05$ according to Turkeys' HSD.

6.3.2 Correlation among leaf mineral content

Correlations among leaf mineral contents were positive and significant ($p<0.05$) among all minerals except Mn, Cu and Na (Table 6.2). However, these three minerals correlated significantly ($p<0.05$) with each other.

Table 6.2: Correlation matrix for leaf mineral nutrient traits of colchicine treated *C.olitorius* plants

c	**N**	**P**	**K**	**Ca**	**Mg**	**Na**	**Al**	**Zn**	**Mn**	**Cu**
P	**0.775**									
K	**0.954**	**0.819**								
Ca	**0.972**	**0.781**	**0.973**							
Mg	**0.921**	**0.777**	**0.967**	**0.931**						
Na	0.118	0.474	0.198	0.131	0.112					
Al	**0.942**	**0.708**	**0.918**	**0.950**	**0.929**	-0.003				
Zn	**0.961**	**0.781**	**0.938**	**0.952**	**0.929**	0.067	**0.971**			
Mn	-0.285	0.262	-0.216	-0.315	-0.264	**0.701**	-0.409	-0.305		
Cu	-0.328	0.252	-0.254	-0.337	-0.265	**0.630**	-0.364	-0.277	**0.926**	
Fe	**0.963**	**0.791**	**0.923**	**0.941**	**0.916**	0.084	**0.958**	**0.993**	-0.277	-0.264

TS- traits; N- nitrogen; P- phosphorus; K- potassium; Ca- calcium; Mg- Magnesium; Na- Sodium; Al- aluminium; Zn-Zinc; Mn- Manganese; Cu- Copper; Fe- Iron. Values in bold are significant ($p<0.05$).

6.3.3 Principal component and cluster analyses

The first two principal components (PC) were associated with Eigen values > 1 and explained cumulatively 93.078% of the total variation (Table 6.3). The first component accounted for 68.370% of the total variation and was mainly defined positively by N, P, K, Ca, Mg, Al, Zn and Fe. The second component explained 24.710% of the total variation and was positively associated with Na, Mn and Cu.

Table 6.3: Principal component analysis (PCA) of the leaf mineral content of colchicine treated *C.olitorius* plants

Variable	**PC 1**	**PC 2**
N	**0.981**	0.007
P	**0.796**	0.543
K	**0.976**	0.092
Ca	**0.985**	0.000
Mg	**0.962**	0.035
Na	0.100	**0.869**
Al	**0.973**	-0.102
Zn	**0.984**	0.001
Mn	-0.313	**0.922**
Cu	-0.317	**0.893**
Fe	**0.977**	0.022
Eigenvalue	7.520	2.710
Variability (%)	68.370	24.710
Cumulative (%)	68.372	93.078

PC1-2: Principal components 1-2, N- nitrogen; P- phosphorus; K- potassium; Ca- calcium; Mg- Magnesium; Na- Sodium; Al- aluminium; Zn- Zinc; Mn- Manganese; Cu- Copper; Fe- Iron. Significant ($p<0.05$) values are in bold.

The principal component biplot grouped colchicine treatments based on their similarity in leaf mineral content explained by the PC1 and PC2 (Figure 6.1; Table 6.3). All minerals, except Mn and Cu, were positively correlated with the first principal component which accounted for the highest variability. Colchicine treatments were clustered into five groups based on leaf mineral content analysis. Group I with low concentrations (0.025 g l^{-1} and 0.05 g l^{-1}) and shorter durations (two and four hours), as well as Group II with same low concentrations but longer durations (six to ten hours), which were positively associated with PC1 were also closely related to the majority of minerals except Cu, Mn and Na. Group V with high concentrations (0.075 g l^{-1} and 0.1 g l^{-1}) but shorter duration (two and four hours) and including the control were positively associated with PC2 and were also related to Cu and Mn. The remaining groups (Groups III and IV) which had higher concentrations and longer durations were negatively associated with both PC1 and PC2.

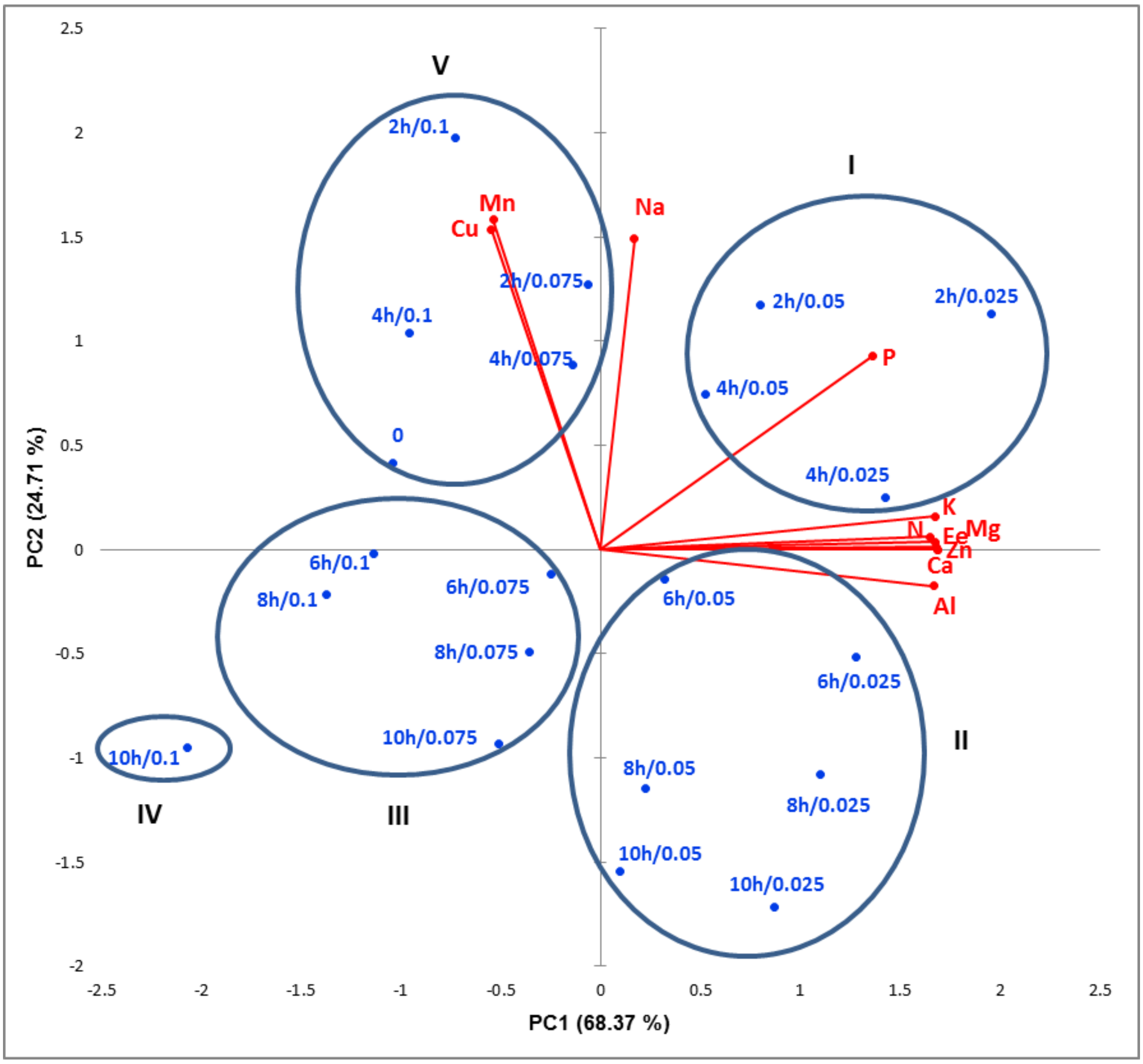


Figure 6.1: Principal component biplot showing variation of colchicine-treated *Corchorus olitorius* plants by mineral nutrient traits.

PC1-2: Principal components 1-2, N- nitrogen; P- phosphorus; K- potassium; Ca- calcium; Mg- Magnesium; Na- Sodium; Al- aluminium; Zn- Zinc; Mn- Manganese; Cu- Copper; Fe- Iron.

The relationship between colchicine-treated *C. olitorius* plants was further illustrated by a dendrogram constructed using the unweighted pair group method of arithmetic means (UPGMA) based on Euclidean distance (Figure 6.2). The dendrogram grouped treatments into two main clusters based on the eleven nutritional traits evaluated. Cluster I consisted of three sub-clusters that mainly contained treatments with longer durations (six to ten hours) combined with a range of colchicine concentrations. Cluster II was also characterised on the basis of shorter durations (two and four hours) and had two sub-clusters. Sub-cluster II-A comprised of the 4h/0.025 g l^{-1}; 4h/0.1 g l^{-1}; 4h/0.1 g l^{-1}; 4h/0.075 g l^{-1}; 2h/0.075 g l^{-1}; 2h/0.05 g l^{-1}; 4h/0.05 g l^{-1} and 2h/0.025 g l^{-1} treatments, while subcluster II-B included plants treated with 0.1 g l^{-1} of colchicine for two hours assembled with the control.

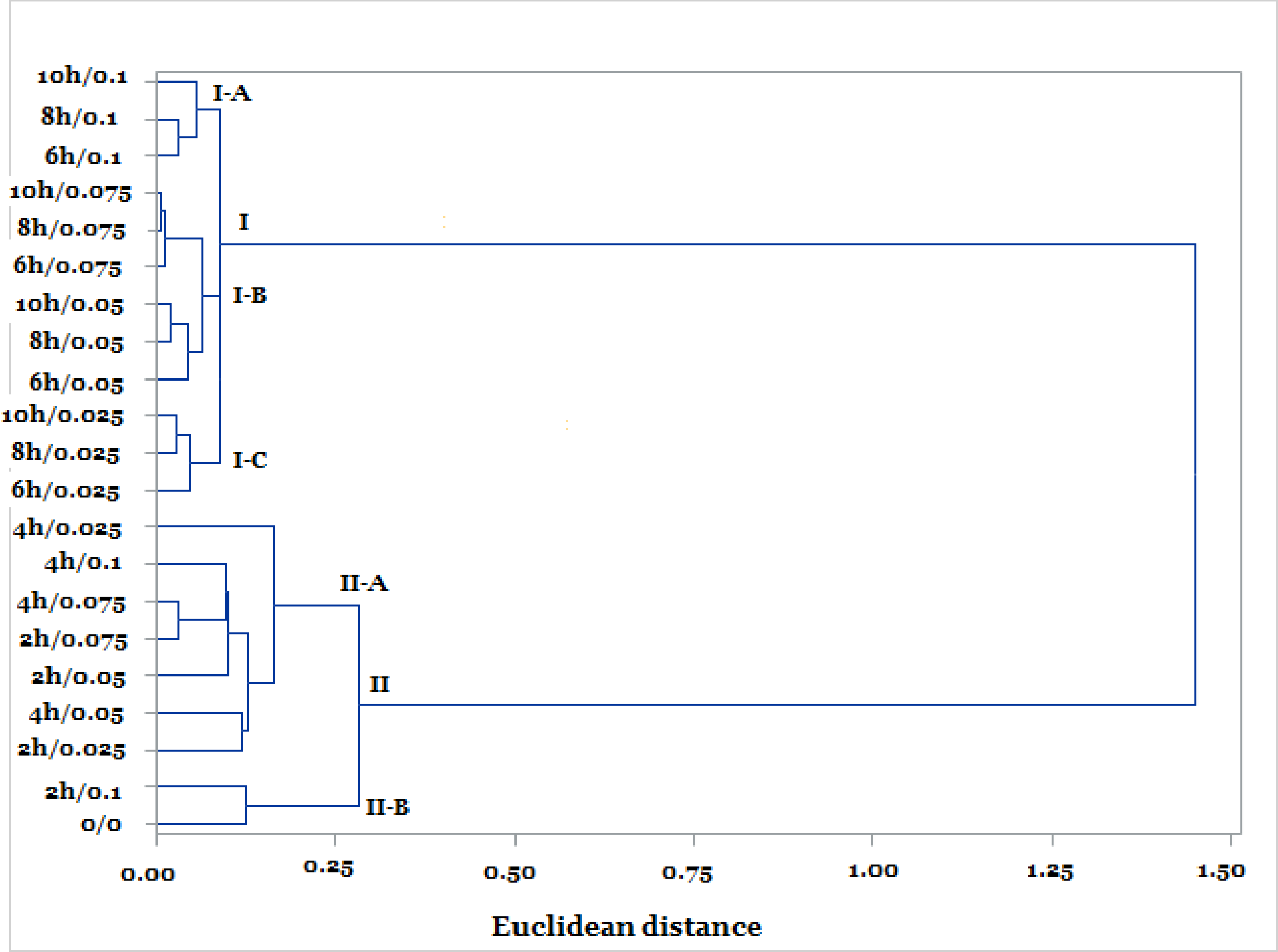


Figure 6.2: Hierarchical cluster analysis dendrogram showing relationship among different colchicine concentration levels and durations of application in *C.olitorius* seeds.

6.4 Discussion

The seed treatment with 0.025 g l^{-1} of colchicine concentration for two hours resulted in plants with significantly ($p<0.05$) higher leaf N, P, K Ca, Mg, Al, and Fe concentrations compared with the control (Table 6.1). These findings concur with the results in *Cannabis sativa* (Bagheri and Mansouri 2015) and *Nicotiana alata* (El-Morsy et al. 2009), where colchicine treatment increased the amount of Ca, Mg and P when compared to their respective untreated counterparts. An increase in the K content of colchicine-treated plants relative to the control treatment was also concurrent with the findings of Ghimire et al. (2016) in *Miscanthus* × *giganteus*.

In general, an increase or decrease in the nutrient content of colchicine-treated plants may be due to an increase in the chromosome number and related gene dose that can sometimes enhance or depress the expression and concentrations of certain secondary metabolites and mineral nutrients in plants (Ghimire et al. 2016). In addition, Ca might be more firmly bonded by pectins in the leaves of colchicine-treated plants, thus enhancing Ca accumulation (Bagheri and Mansouri 2015).

Colchicine treatment significantly reduced the leaf Na and Zn content in *C. olitorious* plants in the present study (Table 6.1). The results of the Na content being significantly lower in colchicine treated plants is contrary to El-Morsy et al. (2009) in *Nicotiana alana* where the lowest Na content was recorded for untreated plants compared with the treated ones. In the present study, the colchicine treatment had no effect on the Cu content in *C. olitorious* plants.

The leaf Ca content was positively correlated with the N, P, K, Mg, Al and Zn content in the current study (Table 6.2). Similar findings were recorded in *Len*s *culinaris* where Ca was positively correlated with P, K and Zn (Karakoy et al. 2012). A strong positive correlation recorded between leaf Zn and Fe in the present study is in agreement with a study on *Vigna unguiculata* (Muranaka et al. 2016). A positive and significant correlation between N, P, K, Mg and Zn was also observed in the present study. A similar correlation was observed in *Vicia faba* where K was positively correlated with P and Zn (Baloch et al. 2014). Also, Fe was positively correlated with N, P, K, Ca, Mg, Al, and Zn in the present study. The positive correlation of Fe with Zn was also reported by Masum Akond et al. (2011) in *Phaseolus vulgaris.* The strong positive correlations observed among the nutritional traits of this study suggest physiological coupling of the accumulation processes of minerals in colchicine-treated *C. olitorious* plants (Ficco et al. 2011). As a result, the improvement of one mineral might simultaneously improve the other. This might be due to the interaction between ions in which chemical properties were equivalently similar, which may compete for the site of absorption, transport and function in plant tissues (Muranaka et al. 2016). Zhao et al. (2017) also pointed out that the positive association of leaf Ca with other minerals in *Triticum turgidum* in their study demonstrated that selection for high Ca concentration may indirectly select for higher levels of other macro- and micronutrients.

In principal component analysis (PCA), PC1 accounted for more than half of the total variability (68.4 %), with N, P, K, Ca, Mg, Al, Zn, and Fe responsible for this high variation among *C. olitorius* plants. Similar results were recorded in *Corchorus* spp. where P, K, Ca and Mg were the major contributors in the nutrient variability in PC1 (Dube 2017). The presence of wide variation among mineral traits described by PC1 suggests that these traits are under genetic control and should, therefore, be liable to genetic improvement (Nyadanu et al. 2014). As a result, these nutrients can be prioritised in breeding selection. PC2 was responsible for 24.710% of the total variability, with Mn being the biggest contributor to this variation.

In the scatter plot, lower colchicine concentrations and shorter durations clustered together; and the same was recorded for higher colchicine concentrations and longer durations. A similar trend was evidenced in the dendrogram, but the clustering pattern was mainly according to treatment durations than both variables.

6.5 Conclusion

Colchicine application mainly at 0.025 g l^{-1} and 0.05 g l^{-1} were associated with enhanced N, P, K, Ca, Al, Fe and Zn. The second cluster also mainly grouped these colchicine concentrations together according to shorter treatment duration, revealing their similar effect on the nutritional status of *C. olitorious* leaves. Therefore, plants treated with colchicine treatment of 0.025 g l^{-1} and 0.05 g l^{-1} were identified as good source of the majority of minerals studied, hence could be selected in further improvement of *C. olitorious*. Even though the duration had an effect on the nutritional content, the effect of concentration was more dominant, this could be the reason why other similar studies have mainly focused on the effect of colchicine rather than both variables. These present findings could be an indication of the success of colchicine treatment on improving the nutritional content in *C. olitorious* plants, with the lowest concentrations being most effective.

CHAPTER 7

General discussion and recommendations

7.1 Rationale of the study

Traditional leafy vegetables are an important source of nutritious food to people in rural communities in South Africa. Many reports highlight the need for investigating the availability, consumption and preference status of TLVs in rural communities (Taleni et al. 2012; Adeniji and Aloyce 2013; Jansen van Rensburg et al. 2014; Konsam et al. 2016). Diversity studies are also important as a preliminary step in breeding of these species for traits such as high leaf yield and micronutrient content (Mavengahama 2013). The KwaMbonambi area in northern KwaZulu-Natal is one area where TLVs are consumed. The overall study included a survey to identify TLVs occurring at KwaMbonambi and to recognize factors affecting their consumption and cultivation. Participatory plant breeding was then used to identify traits of importance to the rural community at KwaMbonambi for preferred TLVs and to use these traits to evaluate 15 *Amaranthus* genotypes. *Amaranthus* was selected because of its popularity as a TLV and because of the availability of unevaluated genotypes. Lastly, the effect of colchicine on the morphology and leaf mineral content of *Corchorus olitorius* was investigated. This TLV was identified in the survey as the third most preferred species by the KwaMbonambi community. Colchicine is able to induce polyploidy in plants which in turn can alter their morphological and nutritional characteristics and create accessions with high variability among them which can be used in breeding programmes. With all this information, there is potential to select species/genotypes which could ultimately be improved and introduced back to the community to increase their access to high-yielding nutritious TLVs.

7.2 Documentation and utilization of traditional leafy vegetables occurring at KwaMbonambi

This research identified 18 species used as TLVs in the KwaMbonambi area. These TLVs are used as food and medicine and are frequently included in the diet of this community. The most predominant method of acquisition was gathering among major crops, particularly by old women. The most preferred TLVs in this area were *Amaranthus hybridus, A. spinosus, A. thunbergii, Bidens biternata and Corchorus olitorius* with *Bidens pilosa, Momordica balsamina and C. olitorius* also used medicinally.

Leaves were the plant part most often consumed and consumption depended on seasonal availability. The respondents reported a decline in the availability of TLVs which they ascribed primarily to drought. Only *Amaranthus* species are cultivated as intercrops among main crops and the survey showed that cultivation of TLVs was hampered by low seed quality.

7.3 Participatory variety selection

Traits selected by respondents of the rural community of KwaMbonambi were used to evaluate 15 *Amaranthus* genotypes. *Amaranthus* was identified as the most preferred TLV genus in the survey as discussed in Chapter 3. Respondents in KwaMbonambi identified mild taste, bigger and numerous leaves, soft texture, and long shelf life as important traits for a good *Amaranthus* or any TLV species in general.

When the 15 *Amaranthus* genotypes were evaluated by the respondents according to the traits they identified, preferred genotypes could be ranked for each trait. Although different genotypes were ranked for different traits, the genotypes ACAT seed fair, Tanzania and AMES-22680 ranked either first or second for most traits. However, since preference and selection for genotypes might vary among different rural communities in the country, there’s a need to conduct similar studies at other locations to further note farmers’ preferences and selections. Such participatory studies will help breeders to identify both traits and genotypes which can be focused on in future breeding programmes.

These promising genotypes selected by the respondents for numerous traits could possibly hasten *Amaranthus* breeding.

7.4 Effect of colchicine treatment on the morphological and nutritional traits of *C. olitorious* plants

The effect of colchicine treatment was first evaluated on *Amaranthus* species. However, the experiment failed due to poor seed germination. A similar study was then undertaken on *C. olitorius* which was the fourth most preferred species in the study area.

The majority of studied morphological traits' values decreased with an increase in colchicine concentration and duration, with the highest values recorded for the control treatment except for leaf chlorophyll content. Among colchicine-treated plants, treatment with 0.025 g l^{-1} and 0.05 g l^{-1} colchicine concentrations, for two and four hours increased seed germination percentage (SG), plumule length (PLL), plant height (PH), leaf number (NL), number of pods per plant (NP/P) and leaf chlorophyll content (LCC) relative to the other treatments where colchicine was applied. However, treatments where colchicine was applied did not significantly increase values for any measured traits relative to the control treatment with the exception of leaf chlorophyll content (LCC). Treatment with 0.025 g l^{-1} of colchicine for two hours significantly ($p<0.05$) increased the N, P, K, Ca, Mg, Al and Fe content in the leaves of *C. olitorious* plants when compared with the control treatment.

An increase in colchicine concentration and duration resulted in *C. olitorious* plants with significantly ($p<0.01$) shorter stems which is a characteristic of dwarfed plants. Dwarfism can be helpful to protect plants against wind and rain by giving them lodging resistance (Jost et al. 2015). Smaller plants can direct more of their growth into the harvestable part and are thus more efficient in terms of photosynthesis and also in fertilizer use (Zhang et al. 2018).

High leaf chlorophyll content is one of the physiological traits that indicate tolerance to water stress in plants (Hadebe et al. 2017). In the current study, an increase in leaf chlorophyll content was also observed in colchicine treated plants which could be an indication that these plants have drought resistance. Drought and seasonal availability were indicated as possible reasons for the decline in consumption of TLVs at

KwaMbonambi (section 3.4.3). Increasing drought tolerance of TLVs could thus increase their availability throughout the year and during periods of drought.

From a crop improvement perspective, this study demonstrated that colchicine treatment resulted in plants with sufficient variation to justify its use in the future to create accessions from which selection can be done. A micronutrient deficiency is still prevalent in many rural areas in South Africa (Jansen van Rensburg et al. 2014). The improvement in the nutritional status of the leaves was therefore of prominence because its consumption will contribute to the alleviation of micronutrient malnutrition among these underprivileged populations. The effect of colchicine concentration and treatment duration on the morphological characteristics and leaf nutrient content was only done over one season in this study due to poor seed germination in the second season.

Increase in the colchicine concentration and treatment duration resulted in a significant ($p<0.05$) reduction in values for most traits measured in this study. This needs to be taken into account in selecting treatment protocols to achieve plants with certain characteristics. For example, treating seeds with low colchicine concentrations and short durations resulted in taller plants, but when treating them with high colchicine concentrations the plants were shorter.

It should be pointed out that determining the ploidy level of plants after colchicine treatment is important. An odd number of chromosomes can result in low fertility (Alam et al. 2011). Since the ploidy status was not tested in the current study, the seed viability is therefore questionable and might influence the future propagation of the seeds from this particular colchicine treatment. In general, the ploidy verification is necessary to be certain if the variations observed between and within various treatments were due to the mutagen or other factors. The fact that plants of treatments with low colchicine concentrations and short treatment durations and those with high colchicine concentrations and long treatment durations were generally clustered together could be an indication that colchicine treatment changed the ploidy level of plants of different treatments and thus resulted in genetic differences among them.

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APPENDICES

Appendix 1.1: Research questionnaire

University of Zululand
Faculty of Science and Agriculture
Department of Agriculture
The questionnaire on the preferred traditional leafy vegetables in the northern of KwaZulu-Natal, South Africa.

Introduction

Good (morning/afternoon/evening), I'm Ngcebo Colile Mncwango and I am conducting a survey on the utilisation of traditional vegetables (imfino) in this area.

Your opinion and knowledge are very important in this research. To obtain reliable, scientific information we request that you answer the questions that follow as honestly as possible. There are no right or wrong answers. All the information you give to us will be kept confidential. You and your household members will not be identified by name or address in any of the reports we plan to write. I also have a form with me that you can sign to show that I have explained the purpose of my research and that you have agreed to be interviewed by me. However if you do not wish to sign it we can proceed with the interview after we have agreed verbally.

Name of the interviewer/ Enumerator	
Date	

1 **Particulars of the area**
1.1 District …………………………………………..
1.2 Municipality ……………………………………………......................................
1.4 Municipal Ward…..……………………………...
1.5 Name of the Location/Area……………………………………………………………
1.6 Name of the Sub-Location/Sub-Area…………………………………...…………

1.7 Name of the Village (Precise place)…………………………………………………

1.8 Induna ……………………………..…………...

2. Particulars of the respondent

2.1 Name of the respondent:

2.2 Gender

1	Male	
2	Female	

2.3 Age range

1	18-30	
2	31-40	
3	41-50	
4	51-60	
5	>60	

3. Preferences, collection, consumption and other uses of traditional leafy vegetables

3.1. Which of the traditional leafy vegetables do you prefer mostly? If more than one, rank them in order of preference.

1.
2.
3.
4.
5.

3.2 Why do you prefer these traditional vegetables?

		TLV 1	TLV 2	TLV 3	TLV 4	TLV 5
1	Larger consumed parts					
2	Better taste					
3	Easily cooked					
4	Easily available					
5	Used to them					
6	Other					

If other, specify…………………………………………………………………………….

3.3 Which part is/are consumed in these TLVs?

1	Leaves	
2	Stem	
3	Shoot tips	
4	Roots	
5	Flowers	
6	Fruits	
7	Seeds	
8	Other	

3.4 Apart from consumption, do you know any medicinal benefits associated with each/ any of the TLV? If yes, name them.

	TLV 1	TLV 2	TLV 3	TLV 4	TLV 5
1					
2					
3					
4					
5					
6					

3.5 What is/are the constraint toward their consumption?

		TLV 1	TLV 2	TLV 3	TLV 4	TLV 5
1	Smaller size of the part consumed					
2	Bitter taste					
3	Seasonal nature					
4	Low shelf life					
5	Other					

If other, specify……………………………………………………………………………….

3.6 How often do you eat these leafy vegetables per week?

1	Once	
2	Twice	
3	Thrice	
4	Daily	
5	Other	

If other, specify……………………………………………………………………………….

3.7 Which food do you eat your traditional leafy vegetable with?

1	Pap	
2	Rice	
3	Bread	
4	Sweet potato	
5	Madumbe	
6	Other	

If other, specify……………………………………………………………………………….

3.8 Which season of the year do you collect it?

1	Summer	
2	Spring	
3	Autumn	
4	Winter	
5	Year round	

3.9 Where do you collect it?

1	Cultivated land	
2	Forest	
3	Grassland	
4	Shrubland	

3.10 Who is responsible for collecting these TLVs in your household?

1	2	3	4	5	6	7	9
Older females	Older males	Young girls	Young boys	Both young and elderly females	Both elder males and young boys	Any family member	Any family member

3.11 If other family members do not collect and eat TLVs what are their reasons?

1	2	3	4	5
It's a woman's job	It's for old people	It is associated with poor people	I prefer improved vegetables like spinach	Other

If other, specify……………………………………………………………………………

3.12 What is the moisture regime of the place where you collect them?

1	Permanent wetland	
2	Intermittent wetland	
3	Dry land	

3.13 Please comment on the population of these vegetables compared to previous years?

Increasing	
Decreasing	
Not changing	
Don’t know	
Never noticed	

3.14 Do you cultivate these traditional leafy vegetables?

1	Yes	
2	No	

3.15 If yes, what time of the year are they cultivated?

1	Summer	
2	Spring	
3	Autumn	
4	Winter	
5	Year round	

3.16 Where do you get seeds of these vegetables?

1	2	3	4	5	6
Collect from own field	From NGOs	From other people’s fields	Purchase	From Dept of Agric	Other (specify)

If other, specify………………………………………………………………………….

3.17 What is/are the selection criteria you used for selecting the seeds?

1	2	3	4
Only larger seeds are selected	Only smaller seeds are selected	Any seeds are selected	Other

If other, specify…………………………………………………………………………….

3.18 What is the method of seed sowing?

1	Broadcasting	
2	Dibbling	
3	Other	

If other, specify…………………………………………………………………………….

3.19 If you grow TLVs, what is the cropping system do you use when growing them?

1	Intercrop stands	
2	Monocrop stands	
3	Other	

If other, specify…………………………………………………………………………….

3.20 Do you apply a fertilizer in your TLV?

1	Yes	
2	No	

3.21 If yes, what type of fertilizer do you apply?

1	Organic fertilizer	
2	Inorganic fertilizer	

3.22 When do you apply the fertilizer?

1	During soil preparation	
2	During planting	
3	During growth	

3.23 If you do not cultivate TLVs why?

1	2	3	4	5	6	7
There is no need	Lack of seeds	Do not eat	I grow the improved ones	Do not eat	They are associated with poor people	Other

If other, specify…………………………………………………………………………………

3.24 If you grow the improved ones which one do you cultivate?

1	2	3	4	5	6
Spinach	Tomatoes	Beetroot	Cabbage	Pumpkins	Other

If other, specify…………………………………………………………………………………

3.25 Why do you prefer them over TLVs?

1	2	3	4	5	6
It seeds are easily available	Used to them	TLV seeds are not available in the market	Larger consumed parts as compared to TLVs	Taste better	Other

If other, specify…………………………………………………………………………………

Appendix 1.2: PVS score sheet

Species	Taste	Odour	Leaf size	Leaf number	Leaf texture	Shelf life	Stem branching	Mean score	Rank
AMES-22680									
Kobie									
AM-fune									
Anna									
Arusha									
A. thunbergii									
ACAT seed fair-									
TOT 2358									
TOT 2266									
TOT 4151									
TOT 8789									
Tanzania									
W6297N									
Tricolor p462179									
Thoyandou									
Score									
Rank									

Likert Rating Scale: P-poor = 1; G-good = 2; F-fair =3; E-excellent = 4. Hedonic scale for colour: 1- dark-green; 2- light-green; 3- yellowish-green; 4- greenish-yellow; 5-yellow. Hedonic scale for the extent of wilting: 1 –none; 2 - slight wilting; 3 - moderate wilting; 4 - severe wilting; 5 -extreme.

Appendix 1.3: Rating scale for wilting of leafy vegetables

Score	Wilting description
1	None
2	Slight: not objectionable
3	Moderate: becoming objectionable
4	Severe: definitely objectionable
5	Extreme: not acceptable under normal conditions

Appendix 1.4: Rating scale for colour of green vegetables

Score	Description
1	Dark-green
2	Light-green
3	Yellowish-green
4	Greenish-yellow
5	Yellow

Source: Kader and Cantwell (2005).

Appendix 1.5: Anova table for seed germination percentage

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Rep	2	1002.77778	501.38889	13.07	<.0001
Plant	1	223.21429	223.21429	5.82	0.0197
Conc	4	27920.83333	6980.20833	182.03	<.0001
Duration	5	36445.83333	7289.16667	190.08	<.0001
Conc*Duration	11	0.00000	0.00000	0.00	1.0000

Appendix 1.6: Anova table for radicle length (cm)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Rep	2	0.75541806	0.37770903	1.92	0.1510
Plant	1	0.45450069	0.45450069	2.31	0.1311
Conc	4	25.95050264	6.48762566	32.99	<.0001
Duration	4	8.03828667	2.00957167	10.22	<.0001
Conc*Duration	12	0.83239333	0.06936611	0.35	0.9767

Appendix 1.7: Anova table for plumule length (cm)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Rep	2	0.02375000	0.01187500	0.27	0.7656
Plant	1	0.00173611	0.00173611	0.04	0.8435
Conc	4	0.84470833	0.21117708	4.76	0.0013
Duration	4	0.64300000	0.16075000	3.62	0.0080
Conc*Duration	12	0.18166667	0.01513889	0.34	0.9797

Appendix 1.8: Anova table for plant height (cm)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Rep	2	1197.897639	598.948819	5.56	0.0049
Plant	1	716.454444	716.454444	6.66	0.0111
Conc	4	2010.375056	502.593764	4.67	0.0015
Duration	4	2235.670000	558.917500	5.19	0.0007
Conc*Duration	12	217.498000	18.124833	0.17	0.9993

Appendix 1.9: Anova table for stem girth (cm)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Rep	2	141.1582875	70.5791438	5.11	0.0074
Plant	1	0.5414507	0.5414507	0.04	0.8434
Conc	4	222.3704837	55.5926209	4.02	0.0042
Duration	4	106.1210283	26.5302571	1.92	0.1114
Conc*Duration	12	12.3227317	1.0268943	0.07	1.0000

Appendix 1.10: Anova table for number of branches

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Rep	2	15.51388889	7.75694444	1.38	0.2560
Plant	1	18.77777778	18.77777778	3.34	0.0702
Conc	4	50.03888889	12.50972222	2.22	0.0705
Duration	4	73.13333333	18.28333333	3.25	0.0144
Conc*Duration	12	11.80000000	0.98333333	0.17	0.9991

Appendix 1.11: Anova table for number of leaves

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Rep	2	495.388889	247.694444	1.36	0.2597
Plant	1	1013.361111	1013.361111	5.58	0.0198
Conc	4	8199.505556	2049.876389	11.28	<.0001
Duration	4	4362.366667	1090.591667	6.00	0.0002
Conc*Duration	12	740.433333	61.702778	0.34	0.9801

Appendix 1.12: Anova table for petiole length (cm)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Rep	2	0.02375000	0.01187500	0.27	0.7656
Plant	1	0.00173611	0.00173611	0.04	0.8435
Conc	4	0.84470833	0.21117708	4.76	0.0013
Duration	4	0.64300000	0.16075000	3.62	0.0080
Conc*Duration	12	0.18166667	0.01513889	0.34	0.9797

Appendix 1.13: Anova table for leaf area (cm^2)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Rep	2	296.237526	148.118763	0.62	0.5413
Plant	1	463.612669	463.612669	1.93	0.1673
Conc	4	1908.237636	477.059409	1.99	0.1008
Duration	4	3731.003655	932.750914	3.88	0.0053
Conc*Duration	12	166.334565	13.861214	0.06	1.0000

Appendix 1.14: Anova table for leaf chlorophyll content

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Rep	2	93.3886056	46.6943028	3.14	0.0467
Plant	1	10.7584000	10.7584000	0.72	0.3964
Conc	4	163.7564156	40.9391039	2.76	0.0310
Duration	4	591.0612133	147.7653033	9.95	<.0001
Conc*Duration	12	64.5536933	5.3794744	0.36	0.9740

Appendix 1.15: Anova table for number of pods per plant

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Rep	2	146.722222	73.361111	5.19	0.0069
Plant	1	7.111111	7.111111	0.50	0.4794
Conc	4	757.772222	189.443056	13.41	<.0001
Duration	4	1625.000000	406.250000	28.75	<.0001
Conc*Duration	12	155.866667	12.988889	0.92	0.5301

Appendix 1.16: Anova table for pod length (cm)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Rep	2	9.12792	4.56396	0.68	0.5077
Plant	1	0.57507	0.57507	0.09	0.7700
Conc	4	16102.85804	4025.71451	601.25	<.0001
Duration	4	8515.14033	2128.78508	317.94	<.0001
Conc*Duration	12	2187.08100	182.25675	27.22	<.0001

Appendix 1.17: Anova table for pod width (cm)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Rep	2	0.00335556	0.00167778	0.88	0.4183
Plant	1	0.00455625	0.00455625	2.38	0.1252
Conc	4	8.79988597	2.19997149	1151.12	<.0001
Duration	4	3.81004167	0.95251042	498.40	<.0001
Conc*Duration	12	1.17083833	0.09756986	51.05	<.0001

Appendix 1.18: Anova table for number of seeds per pod

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Rep	2	519.2639	259.6319	3.52	0.0326
Plant	1	1.5625	1.5625	0.02	0.8844
Conc	4	284106.0264	71026.5066	964.20	<.0001
Duration	4	32046.1167	8011.5292	108.76	<.0001
Conc*Duration	12	32225.0167	2685.4181	36.45	<.0001

Appendix 1.19: Anova table for Nitrogen (N)

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Rep	2	0.00623175	0.00311587	1.44	0.2498
Conc	4	2.07494794	0.51873698	239.14	<.0001
Duration	5	0.26259127	0.05251825	24.21	<.0001
Conc*Duration	11	0.00000000	0.00000000	0.00	1.0000

Appendix 1.20: Anova table for Phosphorous (P)

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Rep	2	0.00892698	0.00446349	2.45	0.0989
Conc	4	0.06209460	0.01552365	8.53	<.0001
Duration	5	0.11329960	0.02265992	12.45	<.0001
Conc*Duration	11	0.00104706	0.00009519	0.05	1.0000

Appendix 1.21: Anova table for Potassium (K)

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Rep	2	0.00419365	0.00209683	0.13	0.8800
Conc	4	1.28099556	0.32024889	19.58	<.0001
Duration	5	0.28315556	0.05663111	3.46	0.0108
Conc*Duration	11	0.09220444	0.00838222	0.51	0.8834

Appendix 1.22: Anova table for Calcium (Ca)

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Rep	2	0.00765714	0.00382857	2.01	0.1469
Conc	4	0.70923810	0.17730952	93.23	<.0001
Duration	5	0.07902976	0.01580595	8.31	<.0001
Conc*Duration	11	0.00317024	0.00028820	0.15	0.9991

Appendix 1.23: Anova table for Magnesium (Mg)

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Rep	2	0.00489841	0.00244921	5.15	0.0103
Conc	4	0.09066794	0.02266698	47.63	<.0001
Duration	5	0.01801627	0.00360325	7.57	<.0001
Conc*Duration	11	0.00689040	0.00062640	1.32	0.2513

Appendix 1.24: Anova table for Sodium (Na)

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Rep	2	3687.254	1843.627	0.94	0.3984
Conc	4	456637.877	114159.469	58.32	<.0001
Duration	5	4100402.199	820080.440	418.94	<.0001
Conc*Duration	11	0.000	0.000	0.00	1.0000

Appendix 1.25: Anova table for Aluminium (Al)

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Rep	2	164.98413	82.49206	2.42	0.1015
Conc	4	31407.74603	7851.93651	230.65	<.0001
Duration	5	5039.32937	1007.86587	29.61	<.0001
Conc*Duration	11	0.00000	0.00000	0.00	1.0000

Appendix 1.26: Anova table for Zinc (Zn)

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Rep	2	8.8571429	4.4285714	0.63	0.5401
Conc	4	632.6666667	158.1666667	22.34	<.0001
Duration	5	217.5000000	43.5000000	6.15	0.0003
Conc*Duration	11	0.0000000	0.0000000	0.00	1.0000

Appendix 1.27: Anova table for Manganese (Mn)

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Rep	2	549.746032	274.873016	3.15	0.0538
Conc	4	2671.688889	667.922222	7.65	0.0001
Duration	5	4915.722222	983.144444	11.26	<.0001
Conc*Duration	11	343.477778	31.225253	0.36	0.9650

Appendix 1.28: Anova table for Copper (Cu)

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Rep	2	1.56857143	0.78428571	0.49	0.6149
Conc	4	13.39733333	3.34933333	2.10	0.0985
Duration	5	21.18583333	4.23716667	2.66	0.0363
Conc*Duration	11	1.66350000	0.15122727	0.09	0.9999

Appendix 1.29: Anova table for Iron (Fe)

Source	**DF**	**Anova SS**	**Mean Square**	**F Value**	**Pr > F**
Rep	2	50.66667	25.33333	0.35	0.7063
Conc	4	22133.56190	5533.39048	76.62	<.0001
Duration	5	5934.01190	1186.80238	16.43	<.0001
Conc*Duration	11	0.00000	0.00000	0.00	1.0000