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resource assessment, utilisation and domestication in Namibia

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ALOE ZEBRINA BAKER: RESOURCE ASSESSMENT, UTILISATION AND DOMESTICATION IN NAMIBIA

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DEDICATION

This thesis is dedicated to:

- My beloved mum, Teresia Palisha Helao and my grandmother Lusia Nelao Mbodje, your prayers and contribution towards my success is invaluable. May the almighty give you strength and bless you forever.
- My husband, El-Salvador Ndeunyema and the twins Elnathan and Eliana Ndeunyema; your understanding, motivation, patience, willingness to share the loneliness and challenging period of my study made us being the strongest team ever.
- *For God so loved the world that he gave his one and only Son, so that whoever believes in him shall not perish but have eternal life.* - John 3:16

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectrometer
AFLP	Amplified Fragment Length Polymorphism
AFPD	African Flowering Plants Database
AHA	American Heart Association
APG	Angiosperm Phylogeny Group
ATNESA	Animal Traction Network for Eastern and Southern Africa
BU	Bangor University
CITES	Convention on International Trade in Endangered Species
DCW	Digital Chart of the World
DNA	Deoxyribonucleic acid
FNA	Flora of North America Association
FZ	Flora Zambesiaca
HPLC	High Performance Liquid Chromatography
ICRAF	International Centre for Research in Agroforestry
IOM	Institute of Medicine
MAWF	Ministry of Agriculture Water and Forestry
MET	Ministry of Environment and Tourism
N$	Namibian dollar
NBRI	Namibia National Botanical Garden Institute
NIED	National Institute of Educational Development
NTSYSPc	Numerical Taxonomy and Multivariate Analysis System
OAC	Ogongo Agricultural College
OM	Organic matter
ON	Optimal Nutrition
OTUs	Operational Taxonomic Units
PCR	Polymerase chain reaction
PNAS	Proceedings of the National Academy of Sciences of the United States of America
ppm	Parts per million

PROTA	Plants Resource of Tropical Africa
RAPD	Random Amplified Polymorphic DNA
SA	South Africa
SBS	School of Biological Sciences
SE	Standard error
SEEN	Sustainable Energy and Economy Network
SEPASAL	Survey of Economic Plants for Arid and Semi-arid Lands
SLS	Sample Loading Solution
SNPs	Single Nucleotides Polymorphisms
SPSS	Statistical Package for Social Sciences
SS	Size standard
TAE	Tris-acetate-EDTA buffer
TBE	Tris Borate-EDTA buffer
UK	United Kingdom
UNAM	University of Namibia
UNDP	United Nations Development Programmes
UoC	University of California
UPGMA	Unweighted pair group method with arithmetic mean method
US$	United States dollar
USA	United States America
WCSPF	World check list of selected plant families
WILD	Wildlife Integration for Livelihood Diversification

ABSTRACT

An integrated study on resource assessment, utilization and domestication of *Aloe zebrina* Baker has been carried out. Field work (socio-economic survey on utilization, management and propagation; ecology and population status; nutritive composition and genetic analysis) was undertaken in Otjozondjupa, Omusati and Ohangwena regions of Namibia. *A. zebrina* material was collected from study areas and investigated at the University of Namibia and Bangor University for genetic diversity and nutritive composition. The socio-economic survey involved 157 respondents interviewed individually and 168 respondents participated in focus group discussion. Participants in study area are knowledgeable of *A. zebrina* species ranging from its existence, status, importance as food, medicine, animal feed, management, possibility of propagation and domestication. Vast knowledge gathered included processing, production and consumption, taste of flower products, demand, supply and cost; nutritional importance, gender role, land tenure including flower collection locations, marketing and income generation. Indigenous knowledge on domestication, propagation and management of the species was also collected. Moreover, trials made on testing propagation of *A. zebrina* species from seeds indicated the potential in growing the species using tested low-cost propagation methods as demonstrated during this study.

The ecology and population status involved collection of climatic data; soil samples and on-site (population status, regeneration and associated plant species) assessment from the study area. Sandy soil was found to be the most preferable soil for growing *A. zebrina* species and too much silt, clay, N and OM in the soil would affect growth and development of the species. Regeneration was affected by over collection of flowers; which does not give chance to seed production, therefore made the species threatened. Similarities in plants associated to *A. zebrina* species observed in the study area, indicated the suitability of these species in the area under study. The nutritive composition analysis involved collection of *A. zebrina* flowers from Otjozondjupa region, which were cooked, sun-dried and analyzed for nutritional composition (protein, ash, fat and dietary fibre), vitamins (nicotinic, pyridoxine, thiamine and riboflavin) and mineral contents (calcium, potassium, magnesium, phosphorus, sodium, iron, manganese and zinc) were determined. The genetic analysis of populations involved DNA extractions and generation of amplified fragment length polymorphism markers (AFLPs) for the 84 individual plants belonging to eight study sites surveyed during the field study. Although genetic diversity study based on 359 bands produced from eight combined primer pairs indicated an 86% polymorphism, which suggested high degree of diversity between *A. zebrina* populations, and the dendrogram constructed showed no significant grouping of *A. zebrina* populations neither by study sites nor by study regions; future research based on similar studies should include further optimization of AFLP molecular markers with adequate repeats samples. However, this study represents a new broadness of integration in the investigation of *A. zebrina* as a resource, its utilization and domestication in Namibia. The socio-economic importance of the species and the utilization of assembled information in the future management of the species are discussed.

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CHAPTER I

GENERAL INTRODUCTION

This chapter provides a brief general overview of the study on the wild plant resource based on succulent plants, with a particular reference to *A. zebrina* in Otjozondjupa, Ohangwena and Omusati regions of Namibia. The chapter has four sections subdivided in subsections as needed. There is a brief background of the state of wild food resources and its significance to the Republic of Namibia, followed by the present study main objectives as well as specific objectives. Study sites selection, criteria for selection, site description as well as a brief summary describing the organization structure of this thesis is presented.

1. 1. Background

The Republic of Namibia lies across the Tropic of Capricorn in southern Africa. It is bordered by Angola and Zambia to the north, Botswana and Zimbabwe to the east, South Africa to the south and southeast and South Atlantic Ocean to the west. The Caprivi Strip, a narrow extension of land in the extreme northeast, connects it to Zambia and Zimbabwe. Namibia covers a land area about 82.4 million ha and it is one of the driest countries in Southern Africa. Namibia's mean annual rainfall ranges from 50 mm in the southwest to 700 mm in the northeastern corner of the country (Hailwa, 1998).

Due to the vast climatic differences in the country, Namibia has broadly been divided into four distinct ecological zones: the desert region which receives less than 100 mm of mean annual rainfall and covers 22% of the land area, the arid region which receives 100-300 mm of mean annual rainfall and covers 33% of the total land area, the semi-arid region with 300-500 mm of mean annual rainfall and 37% of the total land area and the semi-humid region with 500-700 mm of mean annual rainfall and 8% of the total land area. The above ecological zones are divided according to rainfall regimes which have produced different vegetation zones that have been broadly divided further into three major categories: deserts, savannas and woodlands (Hailwa, 1998).

Despite the low rainfall, Namibia has a large variety of useful plant species most of which remain in the wild. Namibia's wild plant resources constitute an important national heritage which provides both economic and environmental benefits to local communities and the nation. The use of wild plant resources as a source of basic needs for local people is an important aspect of multiple-use of land in most parts of Africa including Namibia, ranging from vegetation with low species diversity, high biomass production and flexibility of harvesting, through to vegetation with a high diversity of species, with a multiple of uses and often low resilience to resource harvesting (Cunningham, 1994). Wild plant resources serve as social security for the majority of rural people especially in developing countries. They depend heavily on these resources for their subsistence and livelihood, which necessitate wild plant resources protection, due to the sensitive interface that exists between rural people and wild plant resources. Throughout the world, millions of local people depend on wild plants as a source of food, medicine, fuel and fodder for their livestock (Cotton, 1996; Hamilton, 2006).

Achievement of effective conservation of wild plant resources in a diverse local context remains a major challenge since local people have to be at the heart of plant conservation and where wild plant resources are utilized, this should be done in a sustainable manner. Complexity and costs of managing sustainable use of wild plant resources increase markedly with an increase in the number of uses and resource users. In theory, sustainable harvesting of plants from wild populations is possible, but in practice this is not always possible (Cunningham, 1994).

Conservation of genetic resources of wild plant species, including crop relatives, both *in situ* and *ex situ* is an important aspect of maintaining biological diversity, and the focus has generally been on the small number of species which provide basic food needs, forage and the like (Sandlund, 1992).

The present study has focused on *Aloe zebrina* Baker, (locally known *ekundu*) one of the spotted *aloe* wild plant resources of Namibia that is found growing abundantly in the wild in the southern part of northern regions of Namibia but very scarcely in the extreme north. It has never been domesticated. The flowers of *A. zebrina* are boiled and eaten

straight or pressed into flat cakes and then sun dried and eaten as a delicacy by rural communities of Namibia and Angola. Because of its dietary importance, women in the extreme north of Namibia travel to the southern part of the northern regions (parts of Oshikoto and Otjozondjupa Regions) during the flowering season to harvest the flowers for both sale and home consumption purposes (UNAM Research Agenda, 2003).

A. zebrina was first collected by Welwitsch (Welwitsch 3720, 3721) in July 1854 near Luanda on the Atlantic Coast of Angola and has since been found in abundance almost throughout Angola. In its varying forms, it extends southwards well into Namibia, then eastwards through Zambia and Zimbabwe, and Malawi into Mozambique – almost to the shores of Indian Ocean. The largest population of the species is claimed to occur in Angola (Reynolds, 1966).

Because of the important value of the species as a food and for medicinal use, there is no doubt that domestication of *A. zebrina* would contribute to an improvement of Namibian people's diet as well as their health. As several previous workers have documented, environmental degradation and poor nutrition are some of the major challenges facing Namibian rural communities (NIED, 2009; NEWS-NAMIBIA, 2009; Chotard *et al*, 2006). Domestication of *A. zebrina* may help overcome these challenges and contribute to biodiversity conservation by conserving and restoring the species in Namibian rural communities.

There is, however, a dearth of information on the population status, habitat, genetic diversity and local peoples' knowledge and practices on utilization and management of the species in Namibia. For example, very little information has been documented on its methods of propagation and there are no *A. zebrina* seeds available in the Namibian National Botanical Garden Institute (NBRI) - Gene bank, even for experimental purposes, because most of the flowers are intensively harvested by local people. The first *A. zebrina* seeds were provided to NBRI as a result of the present study.

The present research project was carried out with the aim of assessing the socio-economic importance, population status, habitats, nutritional composition, domestication and management as well as genetic diversity of *A. zebrina* in Namibia.

The study was carried out in partnership with Ogongo Agricultural College (OAC), Namibia, the University of Namibia (UNAM), National Botanical Research Institute (NBRI), Namibia, and Bangor University (BU), United Kingdom.

1.2 The present study

1.2.1 Research objectives

The main objective of the present study was to compile useful socio-economic, chemical and bio-physical information on *A. zebrina* that would enable efficient domestication and facilitate application of appropriate and sustainable management of the species in Namibia.

1.2.2 Specific objective

1. To assess the ecological conditions of the sites where *A. zebrina* occurs;
2. To assess the population status, natural regeneration and associated plant species of *A. zebrina*;
3. To assess the socio-economic importance and management of *A. zebrina* in Namibia;
4. To develop low-cost propagation methods of *A. zebrina* from seeds;
5. To determine the nutritional composition of cooked and dried *A. zebrina* flowers; and
6. To determine the genetic diversity of *A. zebrina* populations.

1.3 Study sites

1.3.1 Study sites selection criteria

The field study was conducted in three northern administrative regions: Ohangwena, Omusati and Otjozondjupa regions, which were selected on the basis of the species availability, types of natural vegetation, rainfall and altitude. Three study sites were selected on communal lands in each of Ohangwena and Omusati regions, whereas in only and two study sites in the commercially farmed Otavi area of Otjozondjupa region.

These gave a total of eight study sites that represented the variation in ethnic groups, land tenure, land use systems (cultivated, uncultivated), types of natural vegetation, rainfall and altitude in Namibia. The field study was conducted between April and September 2007.

1.3.2 Description of the study sites

1.3.2.1 Ohangwena Region

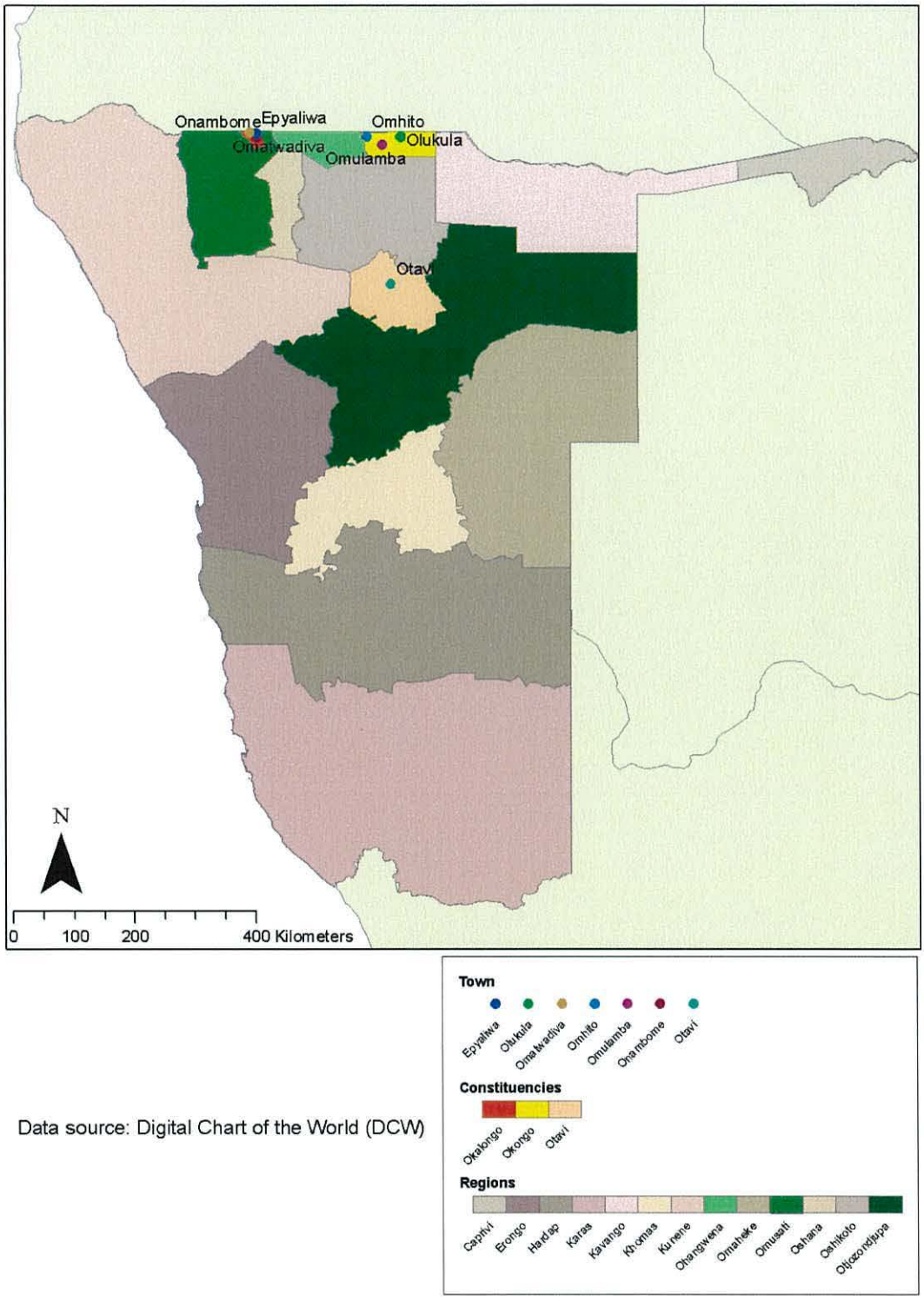
Ohangwena region is a semi-arid area dominated by savanna woodlands consisting of species such as *Pterocarpus angolensis* (Kiaat), *Baikiaea plurijuga, Burkea africana, Terminalia sericea, Combretum collinum, Bauhinia petersiana, Baphia massaiensis, Croton gratissimus* and *Ochna pulchra* (MET, 2000). The annual temperature ranges between 23-34 °C and the annual rainfall varies between 480 mm and 600 mm (Pohamba, 2009) with an altitude of 1157 m.a.s.l (meter above sea level), falls in the range reported by Sheuyange (2005). Rain falls between October/November and March/April. Droughts lasting for several years and frosts that stress the environment occur as rare events (Sheuyange, 2005).

The northern and western parts of the Ohangwena region are the most densely populated of this essentially subsistence agricultural region in which small scale Pearl millet locally known as '*omahangu*' cultivation and cattle rearing form the predominant activities. Although the region depends on rain-fed agriculture, other crops can be established under intensive cultivation. The eastern part of the region possesses good grazing land, but despite the shortage of water and poor communication this renders it uninhabitable at present.

Sheuyange (2005) reported that there is an association between the types of soils and vegetation in Ohangwena region, whereby vegetation is comprised of grass-woodland, bush land and woodland, which are composed of herbaceous cover and grass under storey and sparse tree over storey. The landscape elevation in Ohangwena region ranges from 1100 m.

The three study sites selected in the Okongo constituency of the Ohangwena region were Omhito ya Nanime (17°46' S: 16°97' E), Omulamba (17° 35' S: 17°12' E) and Olukula (17°27' S: 17°29' E) villages (Figure 1.1). Okongo is approximately 840 km north of Windhoek (the capital city of Namibia) and approximately 30 km to the Angolan border.

In the north, Ohangwena borders with Angola: the Cunene Province, except for a small border with Cuando Cubango Province in the far northeast. Domestically, it borders the following regions: Okavango on the east, Oshikoto on the south, Oshana on the southwest and Omusati on the west. The region comprises of ten constituencies: Ongenga, Engela, Oshikango, Ondobe, Eenhana, Omundaungilo, Okongo, Ohangwena, Endola, and Epembe (MET, 2008).



Data source: Digital Chart of the World (DCW)

Figure 1.1 Locations of the eight study sites within Namibia

1.3.2.2 Omusati Region

Omusati region is dominated by *Colosphospermum mopane* woodlands with mean annual rainfall ranging from 300 mm to 455 mm with an altitude of 1000 m.a.s.l (Kolberg, 1998); mean annual average temperature 23.1 °C.

Although mopane tree is the dominant species in the region; Makalani Palms (*Hyphaene petersiana*) are also found abundantly but decrease rapidly westwards from the border with Oshana region. The change in vegetation type reflects ecological conditions forming a natural boundary between the two regions. The northern part of this region is far more densely populated than the south, where grazing resources are of poor quality and water is generally saline. This is also predominantly an agricultural region in which Pearl millet locally known as '*omahangu*' is cultivated successfully (MET, 2008).

Topography of Omusati region is relatively flat without any mountains or hills but an extensive drainage system flowing from Angola southwards into Namibia (Cuvelai delta). Large seasonal pans with relatively shallow, stagnant or very slow flowing water are formed. Soils are mainly white sands with some dark clay in some of the deeper pools. Rainfall normally occurs in the summer months from about December to March. The average annual temperature is about 23°C, whereby an average daily maximum temperature for the hottest month (October) lies between 34 and 36°C and an average daily minimum for the coldest month (July) is between 6 and 8°C.

Natural vegetation has been classified as Mopane Savanna in the western parts, Forest Savanna and Woodland in the eastern parts (Kolberg, 1998; Hillyer, 2006).

According to Rigourd, *et al.* (1999) soils in the North Central Division, where Omusati region is located, are highly heterogeneous characterized by sandy and very poor in nutrient and organic matter content.

The three study sites selected in Omusati region are Onambome (17°47' S: 15°33' E), Epyaliwa (17°27' S: 17°00' E) and Omatwadiva (17°24' S: 15°14' E) villages in

Okalongo constituency, which is approximately 740 km away from Windhoek (Figure 1.1).

In the north, Omusati region borders with the Cunene Province of Angola. Domestically, it borders with the following regions: Ohangwena on the northeast, Oshana on the east and Kunene on the south and west. The region comprises of nine constituencies: Onesi, Tsandi, Uutapi, Okalongo, Oshikuku, Elim, Okahao, Anamulenge and Ogongo.

1.3.2.3 Otjozondjupa Region

Otjozondjupa region is dominated by *Acacia* woodlands, with mean annual rainfall of 600 mm with an altitude ranging between 1310.m.a.s.l. (Heyns, 2008; Jankowitz, 1975) and 1413m.a.s.l. (MET, 2009), and mean annual temperature 20.4 °C.

In the east, Otjozondjupa region borders with the North-West district of Botswana. Domestically, it borders with the following regions: Omaheke on the southeast, Khomas on the south, Erongo on the southwest, Kunene on the northwest, Oshikoto on the north and Okavango on the northeast. Otjozondjupa borders more regions than any other region of Namibia. The region comprises of seven constituencies: Grootfontein, Otavi, Okakarara, Otjiwarongo, Okahandja, Tsumkwe and Omatako.

The two study sites selected in Otjozondjupa region are Otavi municipality plots and Shenga Farm on the outskirt of Otavi Town (19°39' S: 17°20' E) (Figure 1.1). Otavi is about 350 km from Windhoek; it is a small agricultural town in northern Namibia, and also the name of a constituency in Otjozondjupa region. The towns of Otavi, Tsumeb (to the north) and Grootfontein (to the northeast) define an area known as the "Otavi Triangle", also known as the Otavi Mountain land. This geographical entity is sometimes referred to as the "Golden Triangle" or the "Maize Triangle", owing to the cultivation of maize and wheat in the area. The three towns that define the triangle are roughly 60 km from each other (Heyns, 2008; MET, 2008).

Climatic data - rainfall and temperature were collected from Otjozondjupa (Otavi) Oshana (Ondangwa; 17°55' S: 15°57' E), and Omusati (Mahenene) regions, (weather

station locations in brackets). Soil samples were collected from Otjozondjupa (Otavi municipality plots & Shenga Farm) Ohangwena (Omhito ya Nanime, Omulamba & Onghwiyu), and Omusati (Onambome, Ondudu & Omatwadiva) regions, (study sites in brackets).

These data were used in the current study as a reference to climatic requirements and soil growing conditions of *A. zebrina* in Namibian context.

1.4 Thesis organization

The thesis is presented in seven chapters starting with this introduction (Chapter 1) followed by literature review (Chapter 2). The main body of the present study is made up of four chapters: utilization, management and propagation (Chapter 3), the ecology and population status (Chapter 4), nutritional composition (Chapter 5), genetic diversity (Chapter 6). Finally, a chapter with a general discussion, conclusions and future directions (Chapter 7) concludes the thesis.

CHAPTER 2

ALOE ZEBRINA BAKER: LITERATURE REVIEW

This chapter compiles and reviews the state of knowledge as a background for the different aspects of the present study. In the preceding chapter, however, a brief state of knowledge will be noted on the start of every chapter as a supplement to what has been reviewed in this chapter. This chapter is divided into three main sections, which are further subdivided as accordingly. Whereby in the first place the taxonomy and systematic of the species was presented, followed by *Aloe* as a genus, species description in terms of vegetative and reproductive features, habitat, plant size and altitude range, life processes, species that are similar to *A. zebrina* including hybrids, utilization as food, medicine and other economic values. The last part of the chapter discussed domestication and management, geographical distribution and range as well as ecological requirements of the species.

2.1. Taxonomy and systematic

Aloe zebrina Baker '*zebrina* means striped' is a perennial succulent plant commonly known as 'zebra-leaf aloe' or 'spotted aloe' belongs to the Asphodelaceae. The following synonyms of *A. zebrina* are listed in the literature: i) *A. platyphylla* Baker; ii) *A. lugardiana* Baker; iii) *A. constricta* Baker; iv) *A. ammophila* Reynolds; v) *A. komatiensis* Reynolds; vi) *A. laxissima* Reynolds; vii) *A. lettyae* Reynolds; viii) *A. vandermerwei* Reynolds; ix) *A. transvaalensis* Kuntze, x) *A. transvaalensis var. stenacantsee*; xi) *A. bamangwatensis* Schönland; xii) *A. angustifolia* Groenew and xiii) *A. baumii* Engl. & Gilg. (SEPASAL, 2007; WCSPF, 2007; Reynolds, 1974, Jankowitz, 1975; Flora Zambesiaca, 2001). However, the present accepted scientific name of the species is *Aloe zebrina* Baker.

Asphodelaceae is the botanical name of a family of flowering plants, one of many subfamilies into which some classification systems subdivide the Liliaceae. Such a family has not been recognized by all taxonomists and as the result the circumscription of the family has varied. According to Watson and Dallwitz (2006), the Angiosperm

Phylogeny Group (APG) II system of 2003 does not recognize this family as such, but allows it to be segregated from the family Xanthorrhoeaceae, as an optional segregate. As far as APG II accepts this family, it is placed in the order Asparagales, in the clade monocots. This is a slight change from the (APG) I system of 1998, which did firmly accept such a family.

The taxonomic literature of *A. zebrina* is complicated and sometimes confusing. *Aloe zebrina* Baker has been reported in different families; Aloaceae (SEPASAL, 2007; Flora Zambesiaca, 2001), Noctuoidea, (Flora of North America, 2008), Liliaceae (Von Koenen, 1996) and Asphodelaceae (African Flowering Plants Database, 2008; Gateway-Africa, 2005; Leger, 1997). However, the APG II system (2003) has reported that genus *Aloe* was finally placed in the Asphodelaceae family, which includes over a dozen genera, totaling some 800 hundred species, and the best known genus is *Aloe*. The Asphodelaceae family is native to Africa and the Mediterranean basin to Central Asia, with one genus *Bulbinella* in New Zealand. The greatest diversity reported occurs in South Africa.

A. zebrina belongs to a group of aloes called spotted or maculate aloes, which is characterized by relatively small, usually stemless rosettes, spotted leaves and swollen flower bases (PROTA, 2008).

2.2. *Aloe* as a genus

Aloe is a very popular genus of succulent plants occurring naturally in Africa. It contains about 330 species of which about 275 occur in mainland Africa, 40 in Madagascar and 15 in Arabia. The genus is native to Africa and is common in South Africa's Cape Province and the mountains of tropical Africa and neighboring areas such as Madagascar, the Arabian Peninsula and the islands off Africa (SEPASAL, 2007; PROTA, 2008; Van Wyk, 1996, Reynolds, 1966). The genus is found almost throughout the African continent south of the Sahara Desert, except for the moist lowland forest zones and the western end of West Africa (Reynolds, 2004) (Figure 2.1).

The chromosome complement of the genus *Aloe* has long been considered to be exceptionally stable. All species has been reported to have a single basic chromosome

number (x = 7) and a large, strongly bimodal karyotype always comprising of 3 short chromosomes and 4 much longer ones in the haploid set. Polyploidy is now known to be more frequent than was believed earlier, with a record of triploidy (2n= 21) and several of tetraploidy (2n=28) added recently to the single well-known example of hexaploidy (2n=42) in the genus (Brandham and Doherty, 1998; Brandham, 2004).

Flowers of almost all *Aloe* species are diurnal, tubular, brightly coloured red or yellow, unscented and produce abundant nectar. Sun-birds have been noticed being frequent visitors to *aloe* flowers in the field, notably in African gardens. In addition, bees are also reported visited Aloe flowers, especially in South Africa. The flowering of aloes is reported as important in apiculture, although the report claimed that the nectar and pollen of some *Aloe* species can affect the behavior of bees, making them vicious. Other animals such as baboons and wasps are also reported as pollinators (Newton, 2004).

Most aloes have a rosette of large, thick, fleshy leaves. The leaves are often lance-shaped with a sharp apex and a spiny margin. *Aloe* flowers are tubular, frequently yellow, orange or red and are borne on densely clustered, simple or branched leafless stems. Many species of *Aloe* are seemingly stemless, with the rosette growing directly at ground level, other varieties may have a branched or un-branched stem from which the fleshy leaves spring. They vary in colour from grey to bright green and are sometimes stripped or mottled (Flora of North America, 2007).

Vogler and Ernst (1999) have reported on the role of aloes in alternative medicines including home first aid role. Both translucent inner pulp and yellow exudates from wounding the *Aloe* plant is used externally to relieve skin discomfort and internally as a laxative. They further reported that some *Aloe* species have also been used for human consumption, for example drinks made from or containing chunks of *Aloe* pulp are popular in Asia as commercial beverages and as a tea additive; this was notably true in Korea.

Figure 2.1 Geographical distribution map of the genus *Aloe*, showing the number of species in each country (adapted from Reynolds, 2004).

2.3 Description of *Aloe zebrina*

2.3.1 Vegetative features

Aloe zebrina is perennial with either stemless or short-stem (about 30 cm) sometimes growing solitary but usually suckering to form clusters/clump rosette (about 40 cm high) from offsets at the base (Jeppe, 1969; Jankowitz, 1997; Court, 1985; Van Wyk, 1996, Flora Zambesiaca, 2001; Eurica and Rudi, 2005; Faucon, 2005), growing under trees and shrubs (Von Koenen, 1996).

Leaves are about 15-25 cm with irregular white spots in rows. They are borne in a small, dense rosette, varied from linear-lancet to triangular form, from the middle to the top pointed. Leaf length could be either in the range of 15-30, 15-45 or up to 50 cm long and

6-7 cm to 10 cm wide, upper face flat, point gutter form, backside arched (Jeppe, 1969; Jankowitz, 1997; Von Koenen, 1996; Court, 1985; Larsen, 2000, Flora Zambesiaca, 2001).

The leaf colour varies greatly, but tends to be more dull dark green, dull greyish-green, light green to dark green, dark purplish green, purplish green with many white spots arranged in transverse bands on both sides of the leaf marks effectively 'striping' the blade (as the name *zebrina* suggests); leaves are green with whitish streaks, and the species have sharp, orange-red or dark brown thorns along the leaf edges while similar species have thorns along the midrib as well; lower surface similarly but often more distinctly marked, teeth on margins 4-7 mm long, 10-16 mm from each other (Jankowitz, 1997; Van Wyk, 1996; Von Koenen, 1996; Court, 1985; Larsen, 2000; Flora Zambesiaca, 2001, Jeppe, 1969).

Leaf spotting on the upper leaf surface is reported to be always obvious, but varies in intensity on the lower surface (Flora Zambesiaca, 2001; Jeppe, 1969). The sap is honey-coloured and the cut leaf edge dries purplish-black (Jeppe, 1969).

Eurica and Rudi (2005) reported *A. zebrina* as an easy going maculate *aloe* with both surfaces of the leaves having irregular white spots in rows. In natural conditions, the tips of the leaves dry out in summer, and form an attractive curl.

2.3.2 Reproductive features

Inflorescence is much branched with about 1.0-1.5 or 1.5 – 2.0 m high, with sparse, laxy-flowered racemes ranged from pink, dull pink, dull red, bright red or pale red flowers with greenish stripes. Individual flowers are ranged from 3.0-4.0 cm long and have a characteristic swelling at the base (Larsen, 2000; Leffers, 2003; Court, 1985; Van Wyk, 1996; Von Koenen, 1996; Jankowitz, 1997; Jeppe, 1969). ***Bracts*** are 6-15 mm long, linear-lanceolate, scarious, cuspidate, 3-5 nerved. ***Pedicels*** are 6-12 mm long, elongating to 15 – 20 mm in fruit. ***Perianth*** are 25-35 mm long, capsule 25-30 x 14 mm dull-red to pinkish-red or coral-coloured with segment margins paler. ***Seeds*** are 3.5 x 6.5 mm, blackish brown with pale brown wings (Flora Zambesiaca, 2001; Jeppe, 1969).

2.3.3 Habitat, plant size and altitude range

According to SEPASAL (2007) *A. zebrina* grows in various habitats ranging from lowland, hillsides/slopes, outcrops, watercourses, urban anthropogenic landscapes, open grassland, seasonally flooded grassland, woodland, Mopane woodland and wooded grassland to between trees and bushes in parklands. Jankowitz (1975) described *A. zebrina*'s ability to grow on solitary bases but it mostly forms dense -groups growing under or in bushes. Variability of *A. zebrina* species has been documented. For instance, Jeppe (1969) sited the example of *A. zebrina* plants which occurs in the Gauteng province (previously called Transvaal) in South Africa were not found to be typical of the species as compared to the same plant species from Namibia, which show morphological variation (Figure 2.2*)*.

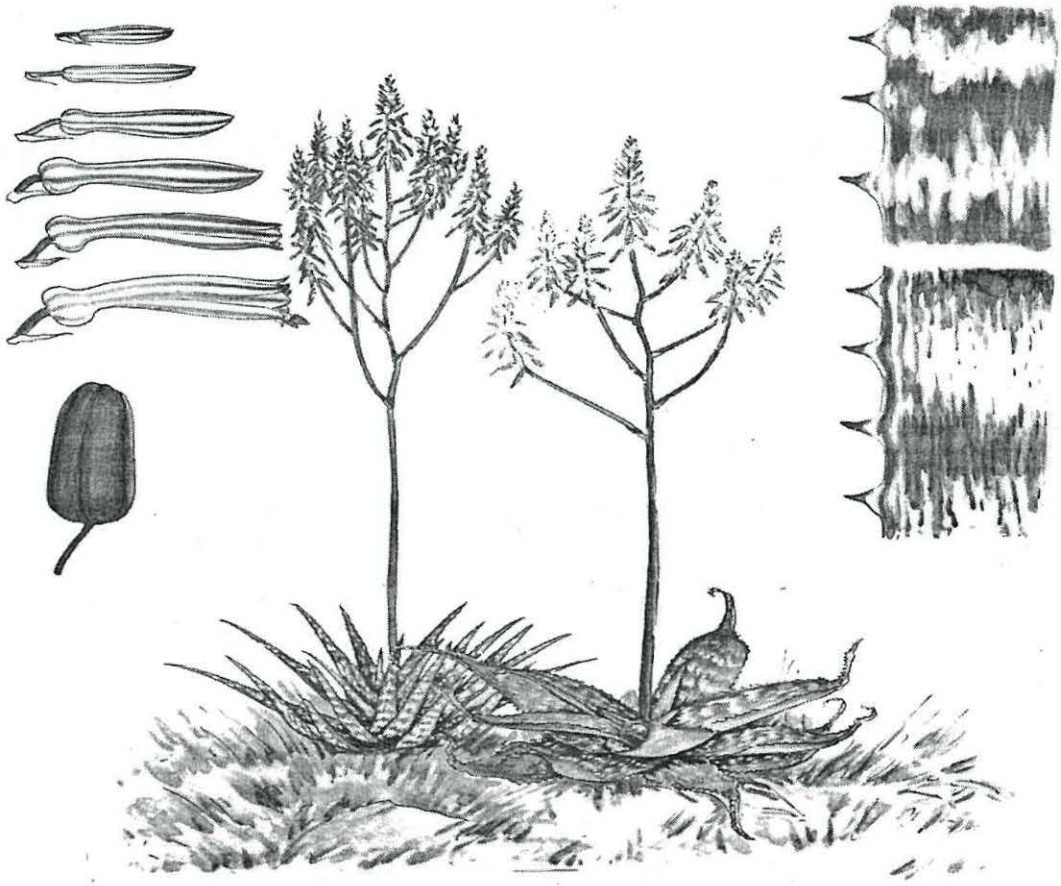


Figure 2.2 Morphological variation of *A. zebrina* species on the left collected from Namibia and on the right occurs in Gauteng - South Africa (adapted from Jeppe, 1969).

Another variation was reported by (PROTA, 2008 and Hyde & Wursten, 2009) that in higher and wetter areas *A. zebrina* plants usually grow singly in grassland, while plants from the lowveld sucker profusely and may form extensive colonies on almost bare-ground. Variation of the species was also stressed by Jeppe (1969) pointing out variability in leaf markings, height and branching of the inflorescence and flowering time (based on the locality). He suggested the main distinguishing characters as being very laxly flowered racemes of about 30-40 cm long, with pedicels of about 6-7 mm long and perianth of about 30 mm.

Moreover, Van Wyk (1996) claimed that due to massive variability in *A. zebrina* in terms of size, leaf spots, inflorescences and flowers, it is still difficult to provide unique characteristics, which has probably led to a number of species names (synonyms to *Aloe zebrina*) reflecting those different characteristics.

Altitude: *A. zebrina* can grow in altitude range of 250 – 1850 m.a.s.l. (Germishuizen & Meyer, 2003; Hyde & Wursten, 2009) and Flora Zambesiaca (2001) documented the altitude range of about 200-1600 m.a.s.l.
Rainfall: The species is reported growing under rainfall range of 500-625 mm per annum (Jeppe, 1969; SEPASAL, 2007).

2.3.4 Life processes

Variation in ***flowering time*** of *A. zebrina* has been reviewed by (Jeppe, 1969; Von Koenen, 2001; Jankowitz, 1997 and Flora Zambesiaca, 2001) who reported that the species flowers from January to May. While Court (1985) declared that flowering occurs from December-April, meanwhile, PROTA (2008) documented that *A. zebrina* flowers during February to April, and Flora Zambesiaca (2001), and then Hyde and Wursten, (2009) claimed that *A. zebrina* flowers between December and March.

Some varieties of *A. zebrina* are reported flowering in June-July (PROTA, 2008; Hyde and Wursten, 2009) and Flora Zambesiaca (2001) stated that flowering of *A. zebrina* occurs in June-August.

2.3.5 Similar species to *A. zebrina*

Larsen (2000) reported that *A. zebrina* is sometimes confused with *Aloe esculenta* locally known as *endobo*, which is not edible. While Rothmann (2004) suggested that from a distance *A. zebrina* may have the general appearance of the young *A. esculenta* but when *A. zebrina* is observed closely, it can be easily identified. Larsen (2000) further claimed that *A. esculenta* is not considered as an edible species in the north central Namibia because their flowers are very bitter and it is only rarely used by people who do not have access to *A. zebrina*. However, Jankowitz (1975), suggested that *A. zebrina* is the only real spotted *aloe* in Namibia, which may not easily be confused with other indigenous species.

The second species reported by Faucon (2005) being similar to *A. zebrina* is *Aloe saponaria* (synonym *A. maculata*). *A. saponaria* has slender and more elongated leaves, about 30 cm long and 10 cm wide, dark green with light green spot leaves with 5 cm long thorns on a lighter green edge.

The third similar species reported by Leger (1997) is *Aloe littoralis* Baker, which is not arborescent, recorded in west Bushmen land of Namibia. Its leaves forms dense, erects rosettes and usually has white spots on a green surface. The inflorescence is about 1.5 m high, branched and bears red flowers during February-March. He further compared the similarity of the two species, that *A. zebrina* Baker is also not arborescent, but its leaves form flatter rosettes and are green and white banded. *A. zebrina* is also reported growing at the sides of an Omuramba and dune areas of Namibia. Its inflorescence, which is also 1.5 m high and much branched like *A. littoralis* Baker's flowers in May and June, and the fourth species similar to *A. zebrina,* was *Aloe greatheadii* Glen (2000).

The fifth species reported being similar to *A. zebrina* was *Aloe parvibracteata* species, and it can be differentiated from *A. zebrina* due to its flowering season, which is only in winter (July) but not in Autumn (March) and the second distinguishing characteristics suggested are that *A. parvibracteata* leaf sap dries golden yellow and not (usually) purple like the case of *A. zebrina*.

In a nutshell, *A. greatheadii* is reported being different from both *A. zebrina* and *A. parvibracteata* in such a way that its raceme (inflorescence) is characterized as subdense to dense, conical to subcapitate terminal raceme. However, all these three species (*A.zebrina*, *A. greatheadii* and *A. parvibracteata*) intergrades in southern tropical Africa and therefore, *A. zebrina* is a member of a difficult and complex group, which would repay intensive detailed investigation.

2.3.6. Hybrids

Natural hybridization has been documented in *A. zebrina* (Jeppe, 1969), especially between *A. zebrina* and *A. littoralis* where both parents grow socially and flower at the same time. It has been claimed that several such crosses were found near Luanda in Angola and elsewhere, however, it has also been reported that *A. zebrina* was regarded to be a useless species to bees due to the fact that its flowers are too narrow to admit bees for pollination. *Aloe angolensis* parentage is thought to be *A. zebrina* x *A. littoralis*, which flowers in July to August. The spots on the leaves of this species are far less regularly arranged than in any other member of this group and it fairly often develops a short erect stem. Flowers characters are intermediate between those of the two possible parent species (*A. zebrina* and *A. littoralis*).

Other cross-breeds reported are *A. zebrina* x *A. swynnertonii*, *A. littoralis* x *A. greatheadii* and *A. parvibracteata* x *A. greatheadii*. Plants of this section are very easily recognized as belonging to this group by the irregular transverse bands of spots on the leaves and conspicuous basal swelling of the flowers. It has been reported that within this section species are very difficult to recognize and therefore, hybridization and other probably continuing evolutionary process make understanding this group a very difficult task. Recognising these hybrids becomes even more challenging after crossbreeding between *A. greatheadii*, *A. parvibracteata* and *A. zebrina* has taken place (Glen, 2000).

2.3.7 Utilization

2.3.7.1 Food value

Throughout Namibia, but especially in the northern part of the country, *A. zebrina*, locally known as *ekundu*, is a very popular vegetable. The flowers of *A. zebrina* are harvested in summer, boiled and eaten straight away or pressed into flat cakes about 15 cm in diameter, and then sun dried on the roof. These cakes, locally called *omavanda* and 'spinach' or 'cabbage' in English, are then stored for use in the dry season when they are soaked in water, boiled, cooked, and other ingredients added for taste and eaten as a delicacy. The preparation and eating of *A. zebrina* flowers is widely practiced especially in Namibia and Angola (Jeppe, 1969; Rodin, 1985; Leffers, 2003).

A. zebrina flowers are sometimes mixed with other vegetable species, especially in areas where the products are in limited supply (Larsen, 2000). Utilization of *A. zebrina* boiled flowers and cakes is also reported along the Kunene River in Angola (PROTA, 2008). The red flowers of both *A. zebrina* Baker and (*Aloe littoralis* Baker, which is a similar species) are gathered when in full flower and cooked together with meat or fish, which result in a red sauce with a tomato-like taste, which the youngsters refers to as 'tomato sauce' (Leger, 1997) and it has been also reported that *A. zebrina* flowers are sucked for nectar by Tswana children (Fox and Norwood, 1982). Besides human utilization of the species Baboons were also reported eat both leaves and flowers (SEPASAL, 2007).

2.3.7.2 Medicinal value

The potential of *A. zebrina* for medicinal value has also been reported. According to Von Koenen (2001) the Herero people of Namibia take a leaf decoction for kidney and bladder problems, including complains related to urination, while leaf sap is used for burns and scalds. Other reported uses of *A. zebrina* are such as dying of *Hyphaene* palm fibres used in basketry weaving to which they give a golden-yellow colour (Jansen, 2005). It was further claimed that like many other *Aloe* species, *A. zebrina* has ornamental value and potential for cultivation in arid to semi-arid frost free locations.

Some studies have reported that the yellow leaf sap of most of *Aloe* species contains important chemical compounds of pharmaceutical importance such as anthrone C-glycosides such as aloin and homonataloin, and the yellow root sap anthranoid aglycones such as chrysophanol (a fast orange-brown colorant) and asphodeline. The roots of *A. zebrina* also contain other important chemical compounds of pharmaceutical importance such as aloesaponarin, aloesaponol and related compounds of the 1-methyl-8-hydroxyanthraquinone pathway. Moreover, isoeleutherol is a unique chemical compound found in the roots of spotted aloes (Van Wyk and Gericke, 2000; Jensen, 2005; PROTA, 2008).

The bitter-juice of many *Aloe* species is used as a powerful purgative and worm expellant, as disinfectant for wounds, and to treat skin problems including conjunctivitis. Whereby, Van Wyk and Gericke (2000) claimed that a decoction of the powdered stem and leaf bases is taken orally twice a day by women after delivery to cleanse the system. Roots and leaves are used traditionally for treating various ailments in both humans and livestock (PROTA, 2008; Rodin, 1985). In one village, the clear leaf gel (applied directly into the eye) was used for treating eye ailments, while in another village, the same aloe gel was used as a treatment for burns and skin ailments, and a few people were reported to use a leaf decoction for stomach problems (Leffers, 2003). Jeppe (1969) documented that in Nova Lisboa (Huambo) in Angola *A. zebrina* leaf sap has been used as a remedy for dandruff (Portuguese *Caspa*).

Amongst *aloes*, *Aloe vera* is the most common and widely used in food, medicine and cosmetic products, hence currently cultivated on commercial scale for extracting aloe juice. It has been used from historic times for its health-supporting properties, and large quantities of aloe gel have been consumed by citizens of just about every country in the world. The species is a rich source of over two hundred naturally-occurring nutritional substances. These components include twenty amino acids, eight enzymes, nine minerals, plant sterols, monosaccharides and polysaccharides, anthraquinones, antioxidants, vitamins A, C, E, B complex, glycoproteins and other substances (Li, 2009; Sapp, 2003).

Other importance of *A. vera* reported by Hunt (2007) that its topical application was reported since the 1930s, whereby a women with a skin burn reported benefited from a topical application of *A. vera* gel and the positive result encouraged trials with others suffering from radiation burns. The plant's wound-healing benefits became scientifically recognized in the 1953s, when a study found it successfully treated radiation lesions in rats. *A. vera* was found to have a great potential beyond topical application, therefore regarded as a 'super' nutrient for a range of health conditions such as immune system modulation, anti-diabetic activity/blood glucose balance, kidney stone prevention, oral/gum health, anti-inflammatory for joint comfort, cholesterol/triglyceride reduction and also antioxidant protection and detoxification. Research conducted to evaluate the anti-hyperglycemic effect of *A. vera* gel and five isolated phytosterols, which were administered to healthy adults for the period of 28 days, found that fasting blood glucose level decreased by up to 64% comparing to control level (Tanaka *et al.* 2006). This suggests that *A. vera* gel and its phytosterols have a long term blood glucose control effect and could be used for the treatment of type 2 diabetes mellitus.

Further, it has been reported that *A. vera* seems to increase absorption of vitamins C and E by means of slowing their assimilation and prolong their concentration in blood plasma, it has been found that *A. vera* increased level of vitamins C and E in the bloodstream by more than 200 percent when consumed together (Vinson, 2005). *A. arborescens* is widely planted and employed as a Japanese folk medicine and health food for oral treatment and exterior use against constipation, can also be made for a variety of food, beverages and cosmetic products (Li, 2009).

2.3.7.3. Other economic values

The economic value of *A. zebrina* species is reported by PROTA (2008) that in north-western Botswana (Ngamiland) and also in its distribution area, the roots of *A. zebrina* are among the main dyes for use in basketry weaving to give a golden-yellow colour, and due to the similarity of spotted aloes, it is suggested that the roots of almost all spotted aloes are suitable as a source of dye. They have been also adopted for wool dyeing by European settlers who introduced the use of metallic mordants such as alum to obtain more intense fast colours. For dyeing *Hyphaene* palm fibres, the roots are collected from the wild, preferably on a small scale, and boiled with the weaving material until golden-

yellow colour is obtained. While in Ethiopia, the leaves of *Aloe* sp. ('sete ret') are chopped and boiled in water with white cloth until it is dyed red (PROTA, 2008).

2.3.8 Domestication and management

According to Reynolds (1950), *A. zebrina* plants in cultivation can reproduce by underground runners. The species can multiply freely through suckers, and can form dense groups (although differences exist between populations). It was reported that its roots can easily be collected on a sustainable basis because the plants can form roots easily; provided they are given a chance to grow again. *A. zebrina* can be raised readily from seeds which are readily available from specialist suppliers (Reynolds, 1950; PROTA, 2008). *Aloe* species are highly decorative and are frequently cultivated as ornamental plants both in gardens and in pots, and are valued by collectors of succulents.

Propagation of the species from both seeds and offsets has been documented, and it is claimed that it does not need special attention in terms of management, especially in warm climate (Eurica and Rudi, 2005). It is reported as very hardy when grown in full sun with minimum water and stands frost better when dry.

In Namibia, however, *A. zebrina* is undomesticated, and although it is known to be drought tolerant, claims have been made that it is a slow growing species and only the flowers are used as food (Larsen, 2000). This author further added that there is little potential for *A. zebrina* cultivation. Moreover, Jeppe (1969) has states that *A. zebrina* is not a good garden subject, because it is hardly attractive with its dull flowers, and leaves are susceptible to rust. However, PROTA (2008) suggested that *A. zebrina* has both ornamental value and high potential for cultivation in arid to semi-arid, frost-free locations.

2.3.9 Geographical distribution and range

According to Rothmann (2004) *A. zebrina* is one of the most widely distributed of all the maculate (spotted) aloes of Southern Africa, and is found in Angola, through Namibia, Botswana and Zimbabwe to Mozambique (Reynolds, 1950; Jeppe, 1969; Van Wyk, 1996; PROTA, 2008; Hyde and Wursten; 2009). Its range extends from Angola on the

shores of the Atlantic in the west, across tropical Africa and 'Portuguese East Africa' up to the shores of Indian Ocean in the east. Van Wyk (1996) claimed that the species is further distributed to northern part of South Africa; while Flora Zambesiaca (2001) documented the species distribution in Zambia and Malawi and also in Swaziland (SEPASAL, 2007) see Figure 2.3. Because of its wide distribution, the species vary considerably. The specific centre for *A. zebrina* appears to be in Angola. The species spreads southwards beyond Kunene River into Namibia (Reynolds, 1950).

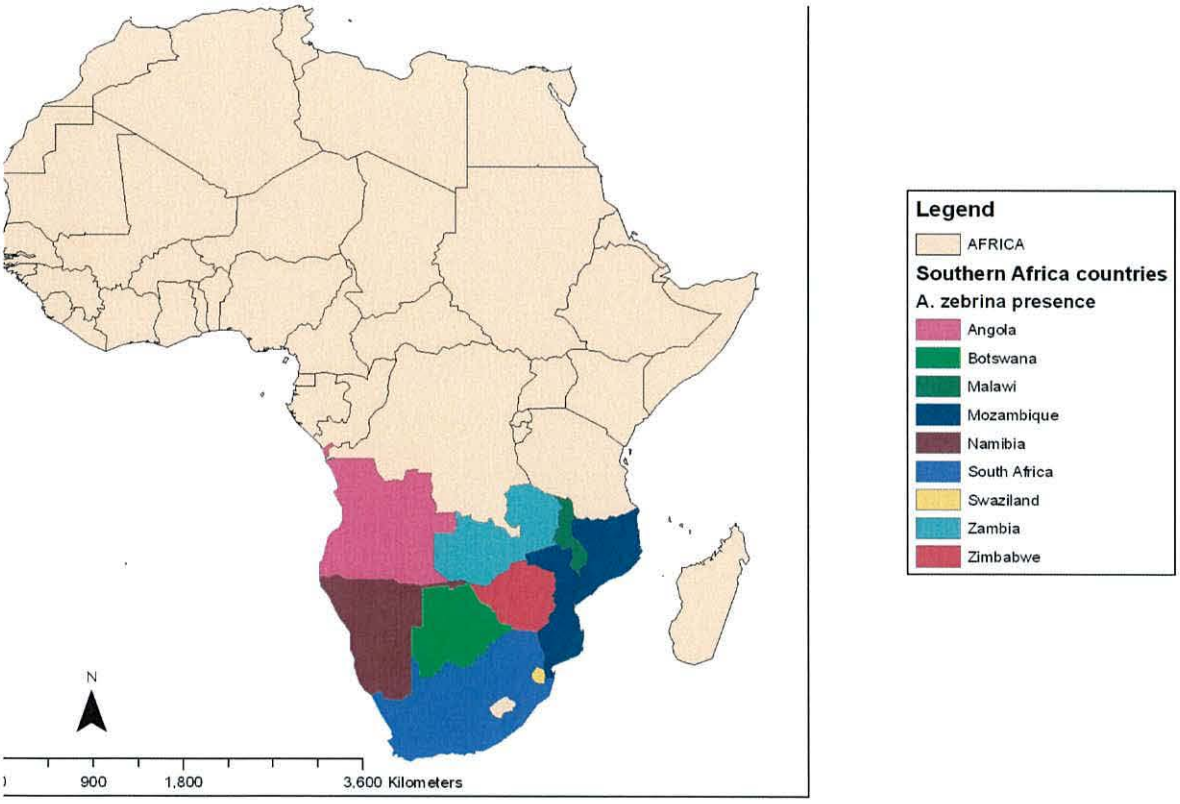


Figure 2.3 *Aloe zebrina* distribution map of Africa

Although Larsen (2000) reported that the species is not evenly distributed in north-central Namibia and the majority of farmers do not have chance to benefit from it due to its scarcity, other reporters documented that in Namibia, *A. zebrina* is distributed through northern regions, Tsumeb, Grootfontein, Otavi, semi-shady northern slopes of the Waterberg to Okahandja and Windhoek; extending eastwards into Botswana (Jankowitz, 1975). The species was also reported being plentiful along the Okavango River where it forms dense-groups (Jankowitz, 1975), while Rothmann (2004) pointed out that the

species is abundant in the Grootfontein region downwards towards Windhoek (the capital city of Namibia) and then east, north and across the Kalahari (Figure 2.4).

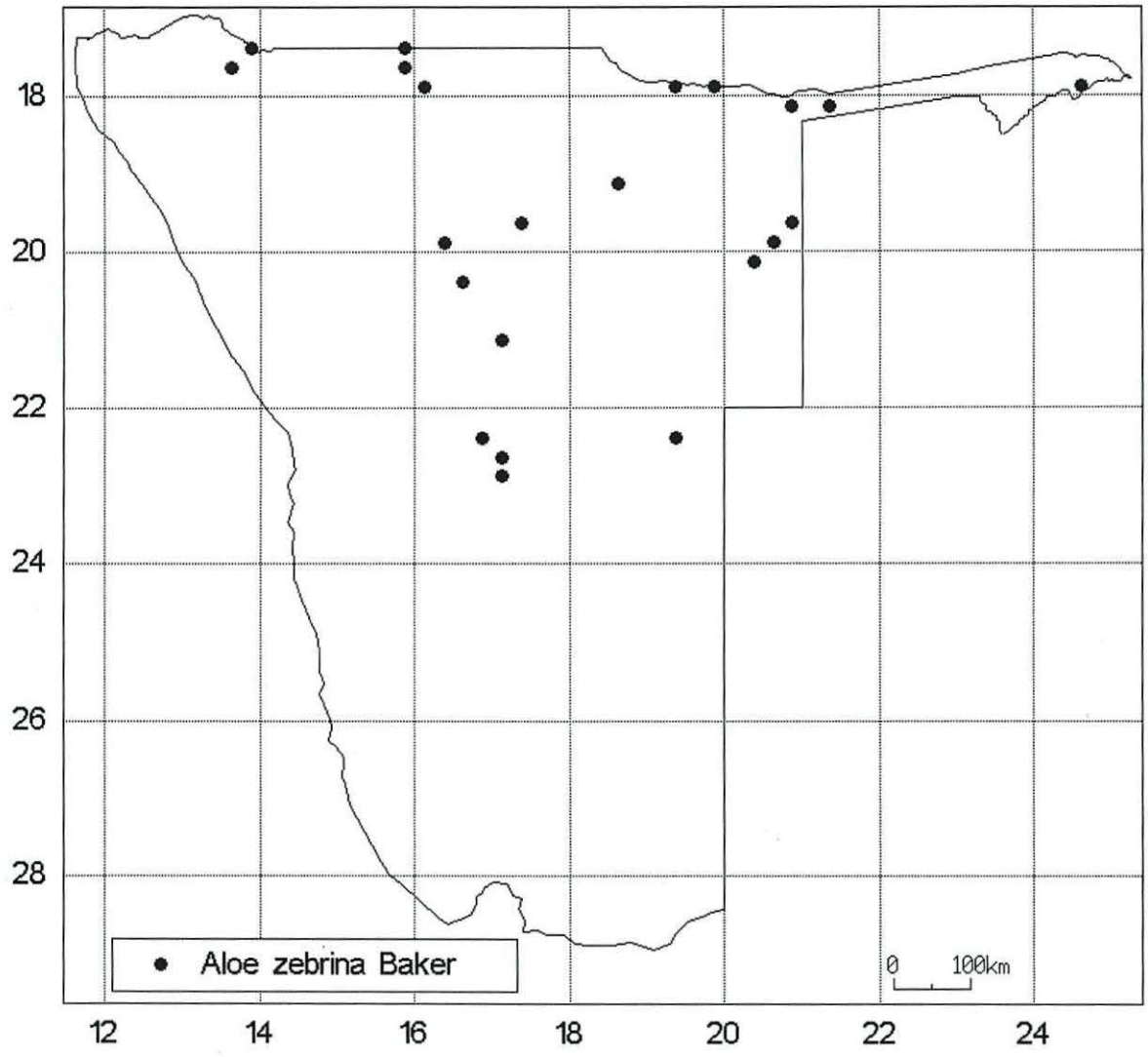


Figure 2.4 *Aloe zebrina* distribution map for Namibia (acquired from NBRI).

2.3.10 Ecological requirements

According to PROTA (2008) *A. zebrina* is found in dry thickets and marshy meadows on river banks. It is not frost tolerant, and may experience some leaf tips destruction if frost

occurs. It prefers minimum average temperature of about 10°C, prefers to grow under full sun and it is a summer grower (Faucon, 2005).

It has been also reported that in the higher and wetter areas, the plants usually grow singly in grassland and have dull coloured, fairly inconspicuous flowers, while plants from the lowveld sucker profusely and may form extensive colonies on almost bare ground and carry brightly coloured flowers (Hyde and Wursten, 2009).

The species' other ecological requirements include soil pH between5.5-6.5, deep, clayey, sand to sandy loam soil and semi-arid climate (SEPASAL (2007; Backyardgardener; 2008). According to SEPASAL (2007) *A. zebrina* grows best under a subtropical, hot and arid climate.

CHAPTER 3

UTILIZATION, MANAGEMENT AND PROPAGATION OF *ALOE ZEBRINA* BAKER IN NAMIBIA

This chapter explores indigenous knowledge on socio-economical importance of *A. zebrina* in terms of utilization, management and domestication of the species. This chapter is composed into four sections subdivided as appropriate. In the first instance, background information is presented, followed by objectives, and then study material and methods composed into data collection based on socio-economic survey and trial on propagation of *A. zebrina* from seeds. The second section presents data analysis; third one presents results on the socio-economic survey as well as on trial on low cost propagation methods at Ogongo Agricultural College, Namibia. The last section discusses the local people's awareness about the species' existence and status as well as harvesting and utilisation of the species.

3.1 Background

Information on the utilization, management and propagation of *Aloe zebrina* is poorly documented. In Namibia, the lack of information is not surprising simply because the species only occurs in the wild and has hardly been domesticated. There have been contradictory reports on the potential of *A. zebrina* for domestication. According to Larsen (2000) *A. zebrina* has got little potential for cultivation. He claimed that although it is drought resistant, it is a slow growing species. Rothmann (2004) also suggested that, although the species is easy to cultivate, it is unimpressive. This was supported by Jeppe (1969), who stated that *A. zebrina* is not a good garden plant because it is hardly attractive with its dull flowers and the leaves are susceptible to rust. On the other hand, (Jansen, 2005) reported that *A. zebrina* has very high ornamental value and therefore has good potential for cultivation in arid to semi-arid frost free locations.

A. zebrina has been reported by several previous workers for being a very important resource used as a source of both human food and medicine by local communities (Leger, 1997; Von Koenen, 2001; Leffers, 2003). The reddish flowers of *A. zebrina* are widely consumed as a delicacy by rural communities in Namibia and Angola. In

Namibia the leaves are also used as a source of water by Bushmen who regard this plant as a major source of water which they obtain by chewing the stalk, but it is only utilized when other more desirable foods are not available. In addition, Tswana children were noticed sucking nectar from *A. zebrina* flowers (SEPASAL, 2007), while Peters *et al.* (1992) reported that baboons also eat the leaves and flowers. The species' utilization for various medicinal purposes has, however, been reported as being very variable with treatments often differing from one village to another (Leffers, 2003). In a study conducted by SEPASAL (2007) the clear leaf gel was mentioned in one village as a valued remedy for the treatment of burns, skin and eye ailments whereby the leaf gel is applied directly to the eye.

Despite *A. zebrina* flowers being a common food delicacy in Namibia, utilization and management of the species are poorly documented. In the present research, therefore, surveys were carried out to licit from local communities their knowledge and practices on the utilisation and management of the species in Namibia.

Overharvesting of *A. zebrina* flowers in combination with uprooting of the species from the wild for several purposes has threatened the species with extinction in Namibia. This requires both *in-situ* and *ex-situ* conservation of the species in order to rescue it from disappearing. Therefore, propagation and domestication of the species is urgently needed. Information on propagation and domestication of *A. zebrina* is, however, poorly documented in Namibia as well as in all Southern African countries where the species occurs. Plantzone (2009) documented that generally aloes are propagated in three ways. The first method is propagating from offsets (young side-plants developing from the base of a mother plant). These are produced most commonly on the stemless, rosette-forming aloes including *A. zebrina*. The second method is by stem cuttings. This is applied to aloes that form more of bush rosettes such as *A. arborescens*. The third method is growing aloes from seeds, which according to Plantzone (2009) would take three to five years before they are large enough to flower. *Aloe* seeds should be sown evenly into sandy, well drained soil and must be covered with a thin layer of sand about 1 mm deep. Germination is reported usually very rapid (Rothmann, 2004), but Plantzone (2009) reported that it can take about three to four weeks to germinate and moisture is required for germination, after which watering should occur only when the soil is completely dry

to avoid rotting and then transplanting of seedlings must be done at 3-4 true leaves. It is further reported that seedlings should be left intact at least for about two seasons or one year before transplantation. It has also been claimed that plants raised from seeds would be mostly hybrids between the various species that flowered together in the growing area such as in the garden (Rothmann, 2004; Plantzone, 2009). There are, however, no reports on propagation and domestication of *A. zebrina* in any of the countries where it occurs. Therefore studies were carried out to develop low-cost method of propagating *A. zebrina* from seeds.

3.2 Objectives

The present research was carried out with the aim of exploring the socio-economic importance of *A. zebrina* and documenting relevant information which may guide future strategies for the conservation, domestication and management of the species in Namibia. The specific objectives were:

a) To assess socio-economic importance of *A. zebrina*;
b) To document local peoples' knowledge and practices on its utilization and management in Namibia; and
c) To develop low-cost propagation method for domestication of *A. zebrina* from seeds.

To address the stated objectives, the following research questions were formulated:

i) What is the socio-economic importance of *A. zebrina* in Namibia?
ii) Do local people manage the resource?
iii) Can *A. zebrina* be propagated?

3.3 Materials and methods

3.3.1. Data collection

3.3.1.1. Socio-economic survey

The socio-economic survey was conducted in all the three study regions: Otjozondjupa, Ohangwena and Omusati regions. In Otjozondjupa region, a questionnaire survey was conducted in Otavi suburb and Shenga farm which is about 10 km south east of Otavi town, Otjozondjupa region. 19 participants were interviewed (Table 3.1). These included an elderly person; a key informant (knowledgeable person) and the rest were farmers. In Ohangwena, household questionnaire surveys were presented to a total of 66 individuals from three villages and in Omusati region 72 individuals from three villages. The sampled informants represented at least 10% of the households in each of the study villages.

Besides the above questionnaire surveys, group interviews were conducted with women and men who harvest *A. zebrina* flowers in each study site (Table 3.1).

Table 3.1 Study sites in Otjozondjupa, Ohangwena and Omusati regions in Namibia

Administrative region	Study site/village	Total number of house holds	Number of households interviewed individually	Participants in group interview
Otjozondjupa	Otavi & Shenga farm	Commercial farms	19	32
Ohangwena	Omhito ya Nanime	33	25	36
	Omulamba	42	23	17
	Olukula	24	18	29
Omusati	Onambome	210	30	11
	Epyaliwa	51	17	21
	Omatwadiva	66	25	22
Total		426	157	168

3.3.1.2. The survey framework

Socio-economic data were collected using questionnaire surveys (Appendix I) and group interview questionnaire (Appendix II) surveys. In all the three regions, individual participants were randomly sampled using simple random sampling technique, whereby the heads of households (whether male or female) was interviewed, husband or wife (if the house was owned by the husband and wife) was interviewed, Youth (23 years and above) were interviewed when parents were not home or no more available and when they live with very old people who could not be interviewed. During Focus Group Discussions (FGDs), participants were divided into groups on the basis of gender and age classes (Figure 3.1, 3.2 and 3.3) men and women, and youth and elderly to avoid domination of one group during discussion. Since respondents were randomly sampled, they ranged from *A. zebrina* flower harvesters to people who may not have much knowledge about the species.

Figure 3.1 Men participants in focus group discussion at Onambome, Omusati Region, Namibia

Figure 3.2 Women participants in focus group discussion at Omhito ya Nanime, Ohangwena Region, Namibia.

Figure 3.3 Youth participants in focus group discussion at Epyaliwa, Omusati Region, Namibia

The interview was conducted in Oshiwambo language especially in Ohangwena and Omusati regions. However, in Otjozondjupa besides Oshiwambo language, Damara/Nama language was used to interview some harvesters of *A. zebrina* flowers in Otavi area, where a translator was employed to facilitate the interview. Each respondent was interviewed once only because time was a limiting factor. The researcher made sure that the questionnaire responses filled during the day were reviewed in the evening for cross checking mistakes and omissions, so that should there be a need for going back then a second visit would be arranged.

3.3.1.3 Trial of propagation of *A. zebrina* from seeds

i) Source of seeds

Since seeds of *Aloe zebrina* were difficult to get or were not available from any source even at the gene bank of the National Botanical Research Institute (NBRI) of Namibia, the researcher had to establish a seed orchard in October 2005 using 40 adult plants that were collected from Omulamba and Omhito ya Nanime in Okongo constituency in Ohangwena region. The 40 adult plants were used in establishing a 10 m x 10 m plot at Ogongo Agricultural College, where the researcher was based. Two years after planting, the plants started to produce seeds which were used in the present propagation experiment. The seed orchard (Figure 3.4) was also the first ever to provide seeds to the seed bank of NBRI as well as to the University of Namibia (seed storage).

Figure 3.4 Established *A. zebrina* seed orchard at Ogongo Agricultural College, Omusati Region, Namibia

ii) Propagation from seeds

Seeds harvested from *A. zebrina* seed orchard plot established at Ogongo Agricultural College (Figure 3.5) were used in this experiment. The experiment was carried out at Ogongo Agricultural College. The aim was to test their germination capacity and ability to grow into full plants (Figure 3.6).

Seeds were sown in polythene pots (up to 5 seeds per pot) at four month intervals. The first sowing was done in October 2006 (10 polythene pots) and the second in February 2007 (20 polythene pots). The variation in the number of pots was due to availability of polythene pots. Survival rate and growth performance of all the plants were done in October 2007, 12 and 9 months after sowing for the first and second sowing experiments, respectively.

Figure 3.5 Seeds harvested from *A. zebrina* seed orchard at Ogongo Agricultural College, Omusati Region, Namibia

Figure 3.6 Fresh seed pods and a seedling grown from seeds harvested from *A. zebrina* seed orchard at Ogongo Agricultural College, Omusati Region, Namibia

3.3.2 Data Analysis

Data processing and analysis was done mainly using the Statistical Package for Social Sciences (SPSS), Minitab 15 and the Microsoft Excel computer software. Before entering data, relevant variables in the questionnaire responses for each question were identified and alternative answers were coded. For closed-up questions, responses were assigned numerical values in the range of 1-3 or more depending on responses. For open-ended questions where respondents provided unstructured answers, responses were assigned string values. By using SPSS the data for all three regions were summarized as frequencies and also percentage among the respondents. Means and standard deviations for open-ended questions were employed, and cross tabulations were used to explore inter-relations of variables for display as tabular summaries.

3.4 Results

3.4.1 Results of the socio-economic survey

3.4.1.1 Species existence and status

A very high awareness of respondents of the existence of *A. zebrina* in their villages was recorded in all the three study regions: Otjozondjupa, Ohangwena and Omusati (Table 3.2).

Table 3.2 Percentage of responses on occurrence of *A. zebrina* in Namibia

Region	Total number of respondents	Yes	No	Do not know
Otjozondjupa	18	100	0	0
Ohangwena	67	98.5	0	1.5
Omusati	72	91.7	5.6	2.8

When respondents were asked whether or not *A. zebrina* has more than one variety, the majority of respondents in all the three regions reported that the species has only one variety (Table 3.3). The highest percentage was recorded in Otjozondjupa (72%; N = 18), followed by Omusati (54%; N = 72) and the least was in Ohangwena (46%; N = 67), however in Ohangwena there was a mixed response as 46% (N = 67) of respondents did not know whether the species has more than one variety or not.

Table 3.3 Percentage of responses on whether *A. zebrina* has more than one variety in Namibia

Region	Total number of respondents	Yes	No	Do not know
Otjozondjupa	18	28	72	0
Ohangwena	67	7.5	46.3	46.3
Omusati	72	19.4	54.1	26.3

Assessment of indigenous knowledge about whether *A. zebrina* species has separate male and female plants showed that a high percentage of respondents from Otjozondjupa (55.6%; N = 17) had knowledge that the species has no separate male and female plants.

However, there was a mixed response from respondents in Omusati with 38.9% (N = 72) who did not know and 33% (N = 72) who suggested that the species has a separate male and female plants, while 27.8% (N = 72) did confirm that *Aloe zebrina* plant has both male and female parts on the same plant (Table 3.4).

Table 3.4 Percentage of responses whether *A. zebrina* species has separate male and female plants in Namibia

Region	Total number of respondents	Yes	No	Do not know
Otjozondjupa	18	22.2	55.5	16.6
Ohangwena	67	3	16.4	80.6
Omusati	72	33.3	27.7	38.8

The results of the assessment whether any change in species abundance in the past 20 years was noticed by the respondents in their villages (Figure 3.7), showed that a decrease in abundance of *A. zebrina* in the past 20 years was noticed by 79% of respondents from Omusati, followed by Otjozondjupa (49%). However, a significant percentage of respondents from Ohangwena observed an increase in the species abundance (42%).

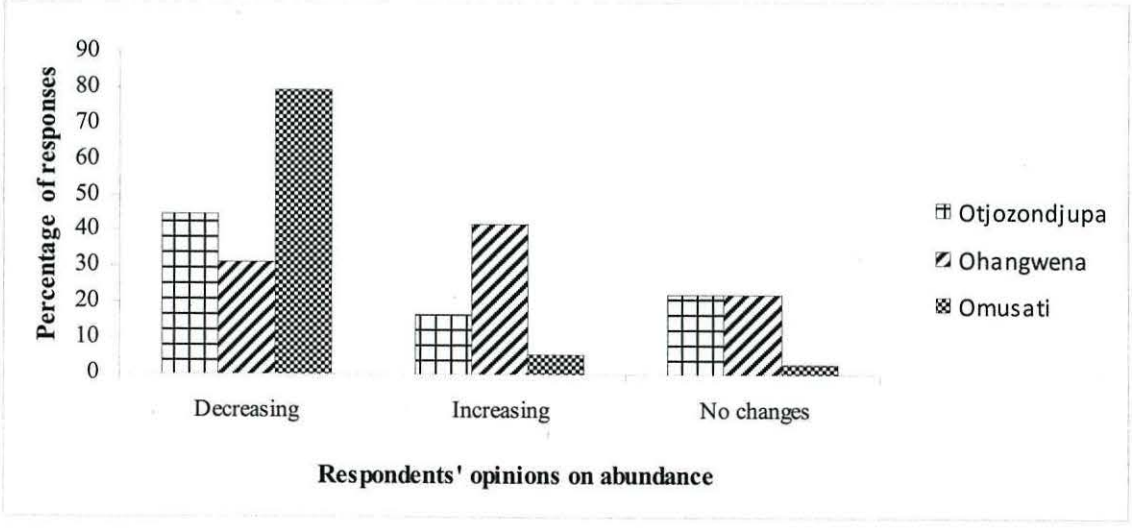


Figure 3.7 Percentage of responses on changes on the abundance of *A. zebrina* 1987 – 2007 in Otjozondjupa, Ohangwena and Omusati regions, Namibia

3.4.1.2 Importance / uses of *Aloe zebrina*

Involvement of respondents in harvesting of *A. zebrina* flowers has been confirmed in all the three study regions, with 100% response in Otjozondjupa, 92.5% in Ohangwena and 80.6% in Omusati region (Table 3.5).

Table 3.5 Observed frequency of responses on involvement in harvesting *A. zebrina* flowers in Namibia

Region	Total number of respondents	Yes	No
Otjozondjupa	18	18	0
Ohangwena	67	62	5
Omusati	72	58	14

However when they were asked if a harvesting permit was required when harvesting *A. zebrina* flowers, there was a difference in response from Otjozondjupa compared to other regions. The majority of respondents in Otjozondjupa (12 of 18) stressed that harvesting permit was always required; in contrast to respondents in Ohangwena and Omusati regions (57 of 67) and (65 of 71), respectively who pointed out that harvesting permit was not a requirement when harvesting *A. zebrina* flowers in their areas (Table 3.6).

Table 3.6 Observed percentage of responses on acquiring harvesting permit when harvesting *A. zebrina* flowers in Namibia

Region	Total number of respondents	Yes	No
Otjozondjupa	18	67	33
Ohangwena	67	15	85
Omusati	72	8	90

Based on the findings shown in Figure 3.8, harvesting time of *A. zebrina* was different in Otjozondjupa (May – September) compared to Ohangwena (January – April). However there was an overlap in the species harvesting time reported in Omusati (February – September).

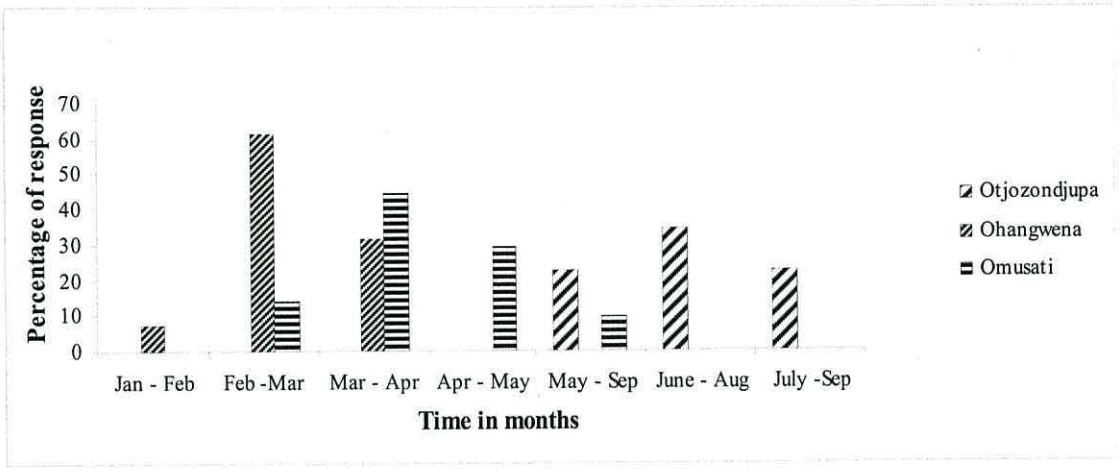


Figure 3.8 Percentage of responses on harvesting date of *A. zebrina* flowers in Namibia

The current study found that there were three main reasons why respondents harvested *A. zebrina* flowers. These included: for domestic consumption, for sale, and for both domestic consumption and sale (Figure 3.9). In the case of Otjozondjupa region, domestic consumption & sale was the major aim for harvesting the species (15 of 18 respondents). While in Ohangwena, domestic consumption was the main factor (57 of 66) with none of respondents harvesting the species for sale. Then, in Omusati, although respondents indicated that domestic consumption (42 of 68) as their major aim, they also harvested the species for both consumption & sale (25 of 68).

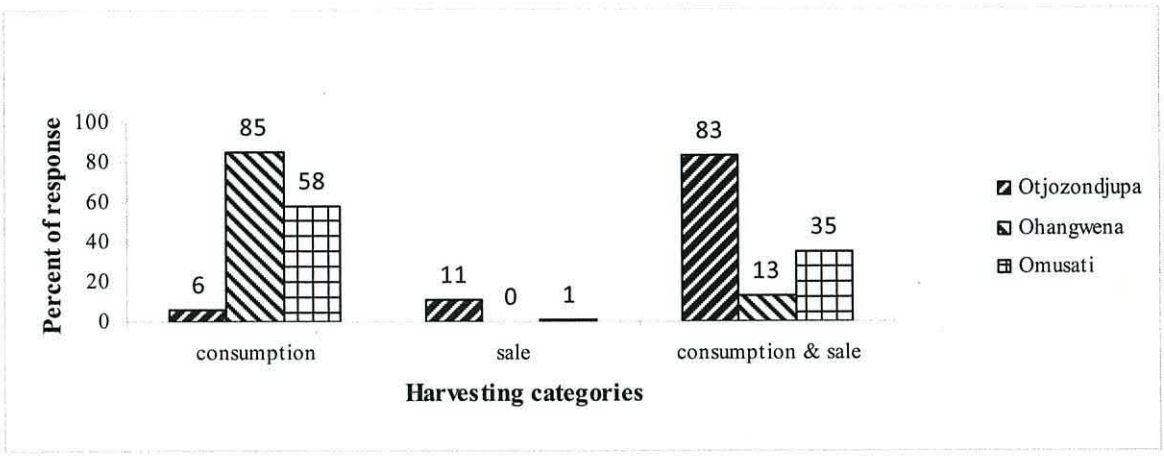


Figure 3.9 Percentage of responses on reasons for harvesting *A. zebrina* flowers in Namibia

When respondents were asked if they selected *A. zebrina* flowers when harvesting, the majority of respondents in all three regions admitted selecting flowers (Table 3.7).

Table 3.7 Percentage of responses on whether participants do select *A. zebrina* flowers during harvesting, Namibia.

Region	Total number of respondents	Yes	No
Otjozondjupa	18	72	28
Ohangwena	67	63	23
Omusati	72	74	16

When further asked why they selected *A. zebrina* flowers during harvesting, the majority in all three regions reasoned that they only selected best mature flowers, because they claimed the more mature the flowers were the sweeter the flower products would be (Table 3.8).

Table 3.8 Percentage of respondents who provided reasoning for selecting *A. zebrina* flowers when harvesting in Namibia

Region	Total number of respondents	reasoning	no reasoning
Otjozondjupa	18	56	44
Ohangwena	67	66	34
Omusati	72	72	28

3.4.1.3 Processing of flower products

According to individual respondents as well as those who were group interviewed, two products (the cake locally known as *evanda* and 'spinach' locally called *oshinyanekela*) are obtained from *A. zebrina* flowers. The methods of processing of the two products are described in Figure 3.10 (a and b).

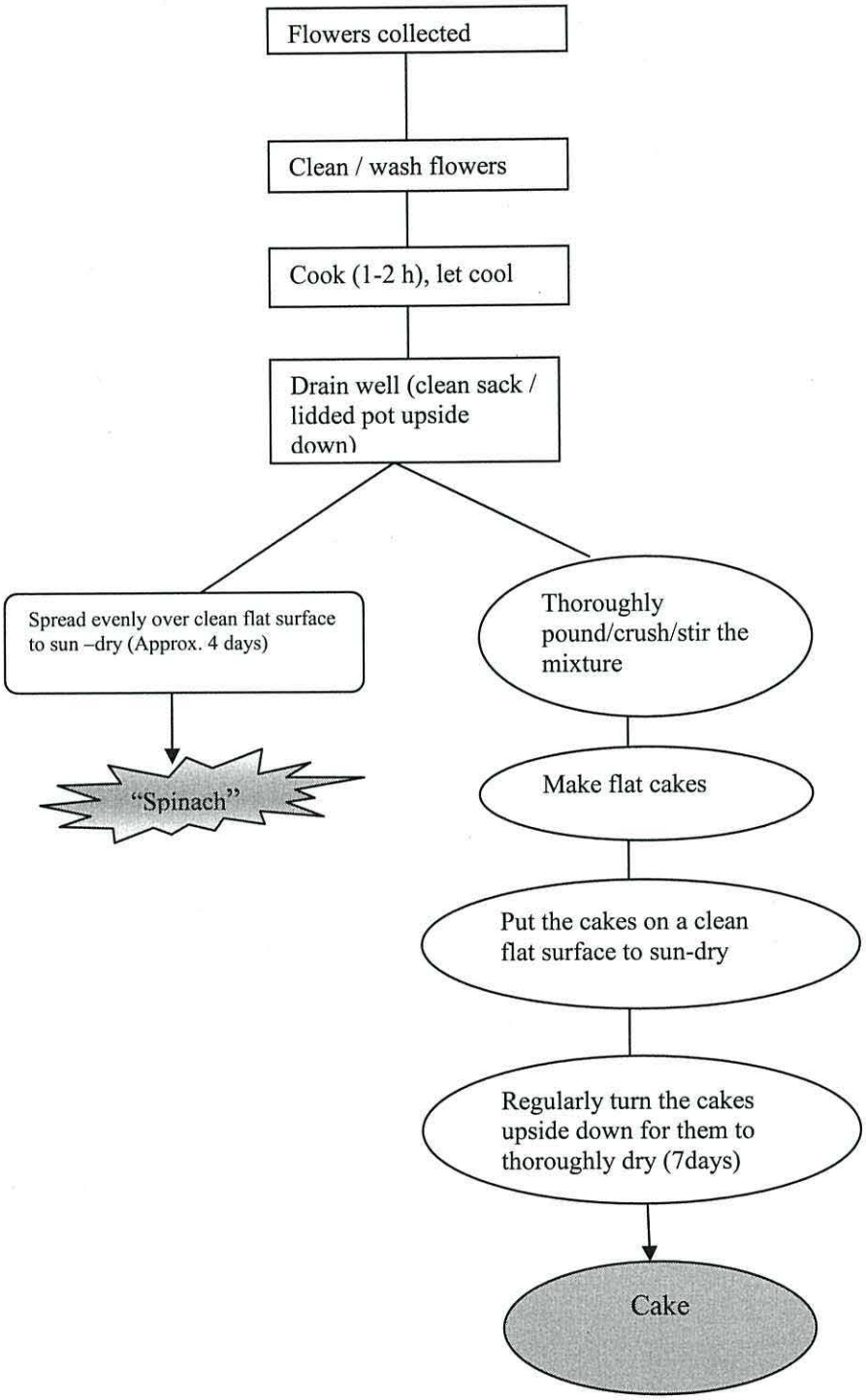


Figure 3.10 (a) *Aloe zebrina* "spinach" and cake production process

Figure 3.10 (b) *Aloe zebrina* "spinach" and cake production process to marketing together with other products

3.4.1.4 Production and consumption

Respondents had good knowledge about the number of cakes that can be produced from a single *A. zebrina* plant. Respondents clearly stated that the amount of flowers produced from a single plant was not enough to make one full cake. However, there was a slightly regional differentiation in how much flowers could be produced from a single plant. The mean number of cakes produced from a single plant was 0.14 ± 0.05 cakes in Otjozondjupa, 0.70 ± 0.10 cakes in Ohangwena and 0.41 ± 0.08 cakes in Omusati region (Figure 3.11).

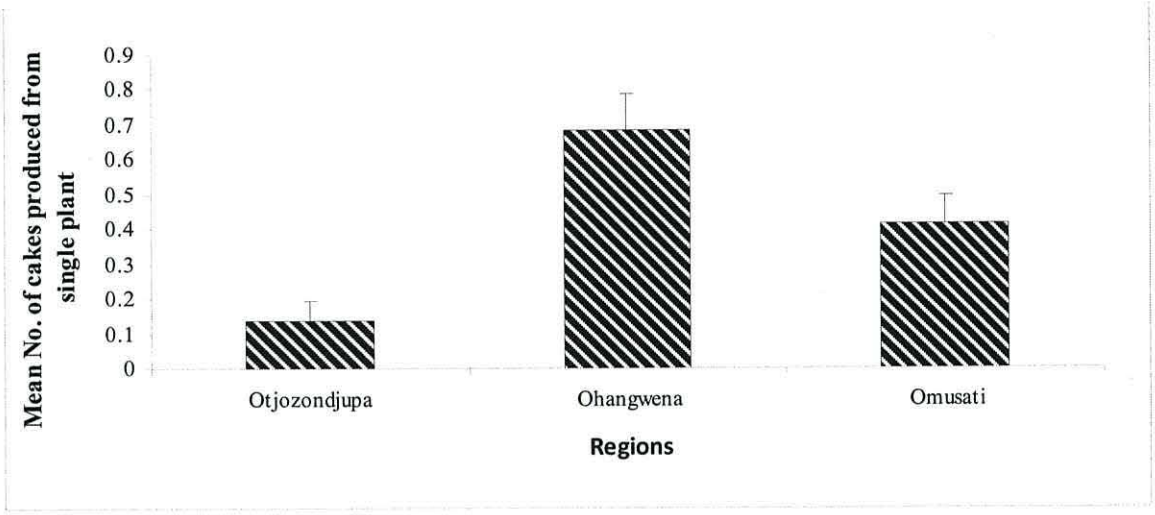


Figure 3.11 Average number of cakes produced from a single *A. zebrina* plant in Namibia

Results on the assessment of consumption of the flower products indicated that Otjozondjupa region had the highest number of both cakes and dried 'spinach' in kg consumed per household when compared with Ohangwena and Omusati regions. The mean number of cakes consumed per household per year in Otjozondjupa was 2645±53.99 and spinach 75±21.98 kg and in Ohangwena it was 35±7.86 cakes and 1.31±1.16 kg spinach (Figure 3.12 and Figure 3.13, respectively).

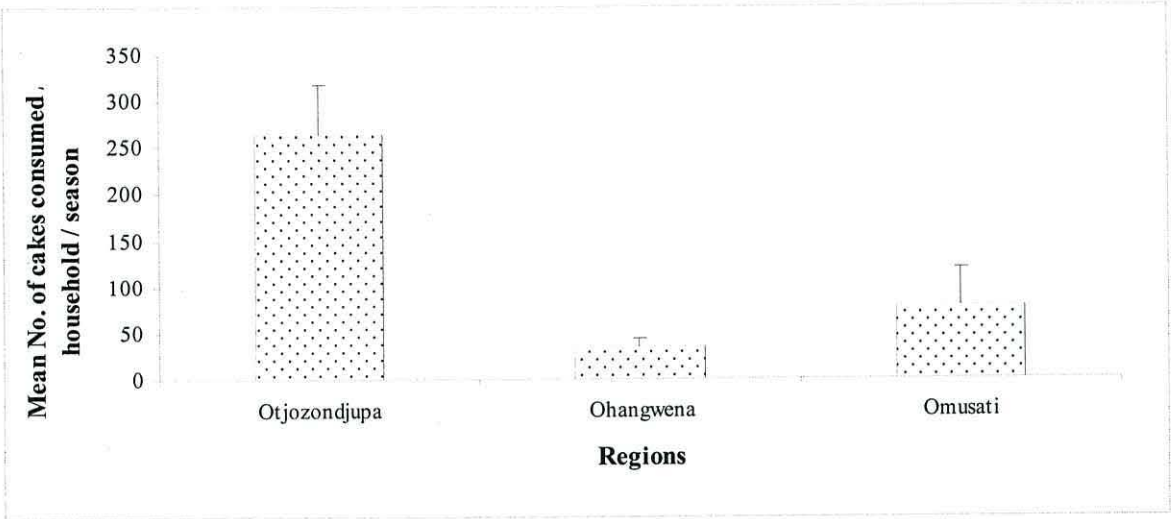


Figure 3.12 Average number of *A. zebrina* cakes consumed per household per year in Namibia

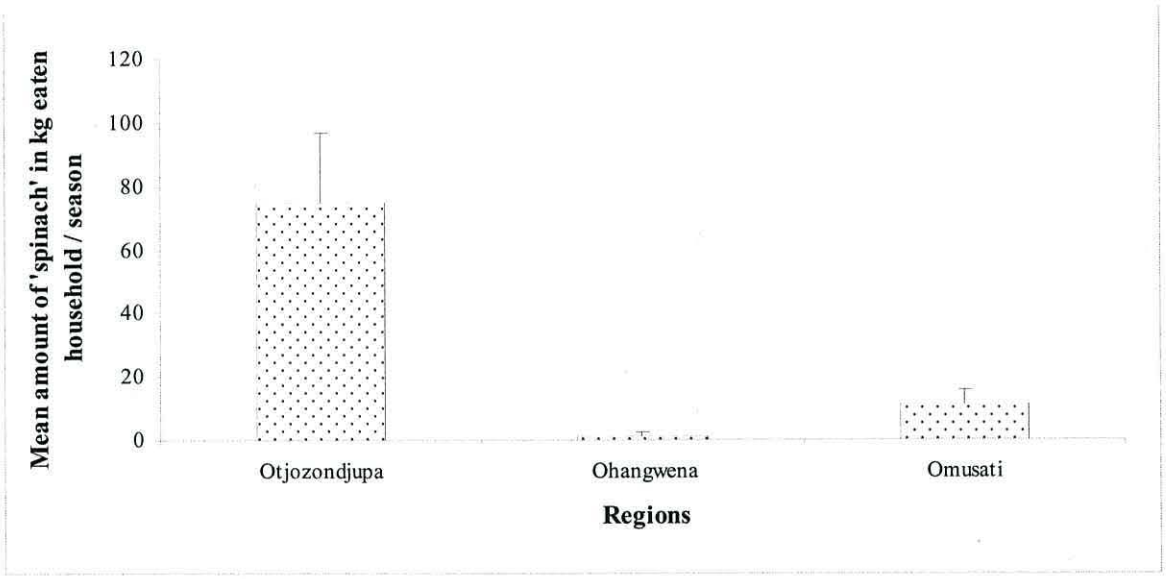


Figure 3.13 Average amount of *A. zebrina* 'spinach' in kg consumed per household per season in Namibia

3.4.1.5 Taste of flower products

The taste of *A. zebrina* flower products was also assessed including factors that could improve the taste of the products. Over 90% of the respondents in all the study areas considered cooked *A. zebrina* flower products to be very sweet as shown in Figure 3.14.

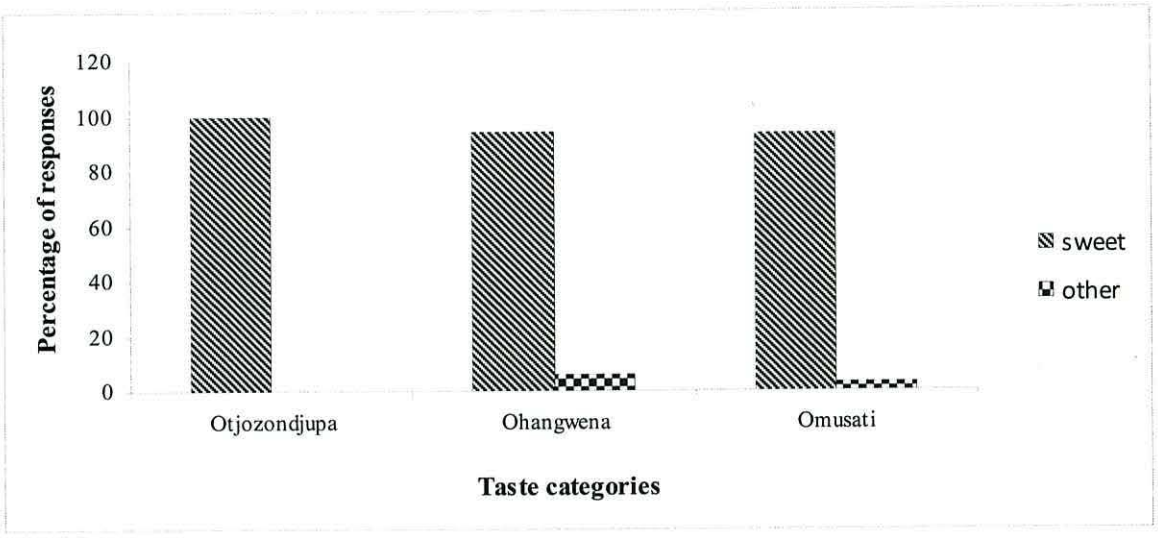


Figure 3.14 Observed frequency of responses on the taste of cooked *A. zebrina* flowers in Namibia

It was stressed by majority of respondents that the addition of other ingredients to influence the taste of *A. zebrina* flower products was not important. Ingredients that could be added to cooked flower products to make them a better delicacy was reported by some respondents (Figure 3.15).

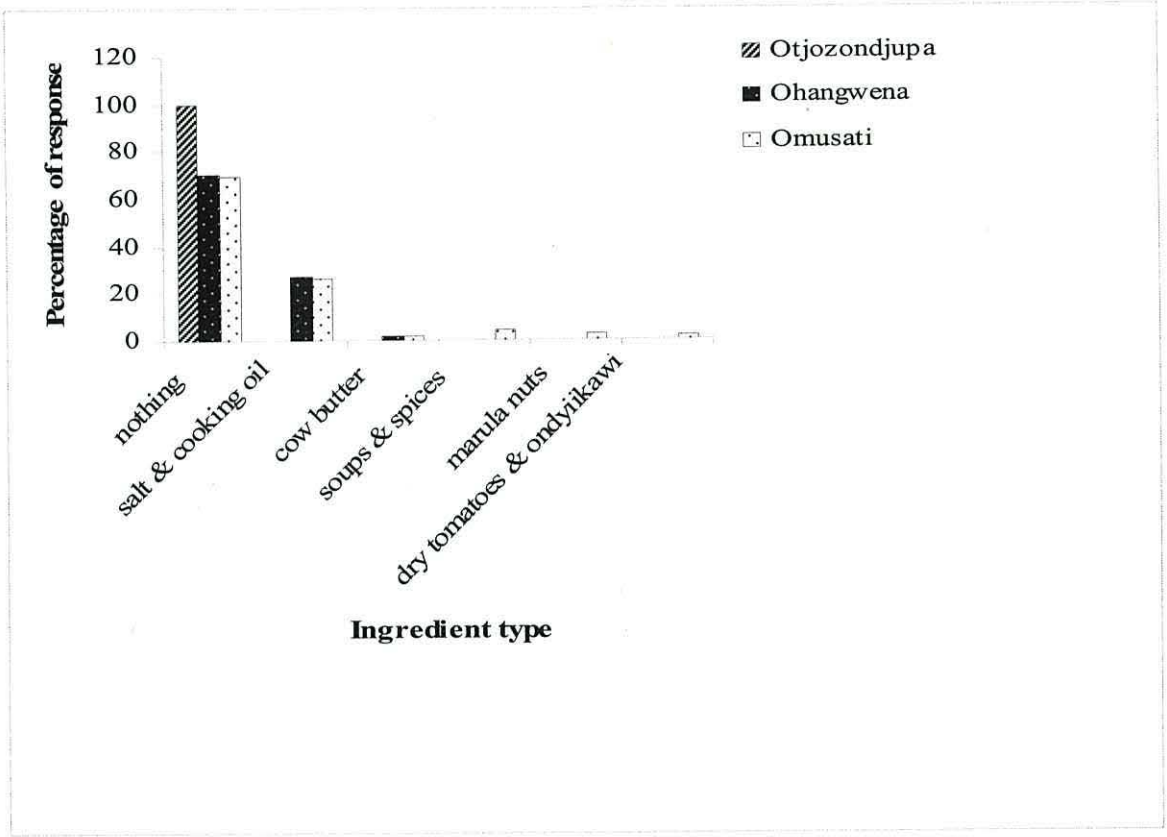


Figure 3.15 Percentage of response on ingredients that influence the taste *A. zebrina* flower products in Namibia.

3.4.1.6 Demand, supply and cost of flower products

Purchase of processed *A. zebrina* flower products was recorded in all three study regions, and the demand of processed *A. zebrina* flower products was generally considered high by over 50% of the respondents (Table 3.9). Highest demand was noted in Omusati region (87.5%), while Otjozondjupa had the lowest demand (33.3%).

Table: 3.9 Percentage of responses on demand and supply of *A. zebrina* flower products in Namibia

	If buying *A. zebrina* flowers products at all			If *A. zebrina* flower products readily available			If demand of *A. zebrina* flower products is high		
Region	**Yes**	**No**	**N**	**Yes**	**No**	**N**	**Yes**	**No**	**N**
Otjozondjupa	33.3	66.7	18	55.6	33.4	16	61.1	38.8	18
Ohangwena	50.7	49.3	67	34.2	64.2	66	31.8	68.1	66
Omusati	87.5	12.5	72	76.4	22.2	71	64.7	35.2	71

Respondents in the study areas also indicated their sources of supply for *A. zebrina* flower products. The study on the supply chain showed that local women vendors were the major suppliers of *A. zebrina* flower products in all the three regions (Figure 3.16). Omusati region was found to have the highest number of supply sources of *A. zebrina* flower products, which was extended to the borders of Namibia and Angola up to Ombadja inside Angola.

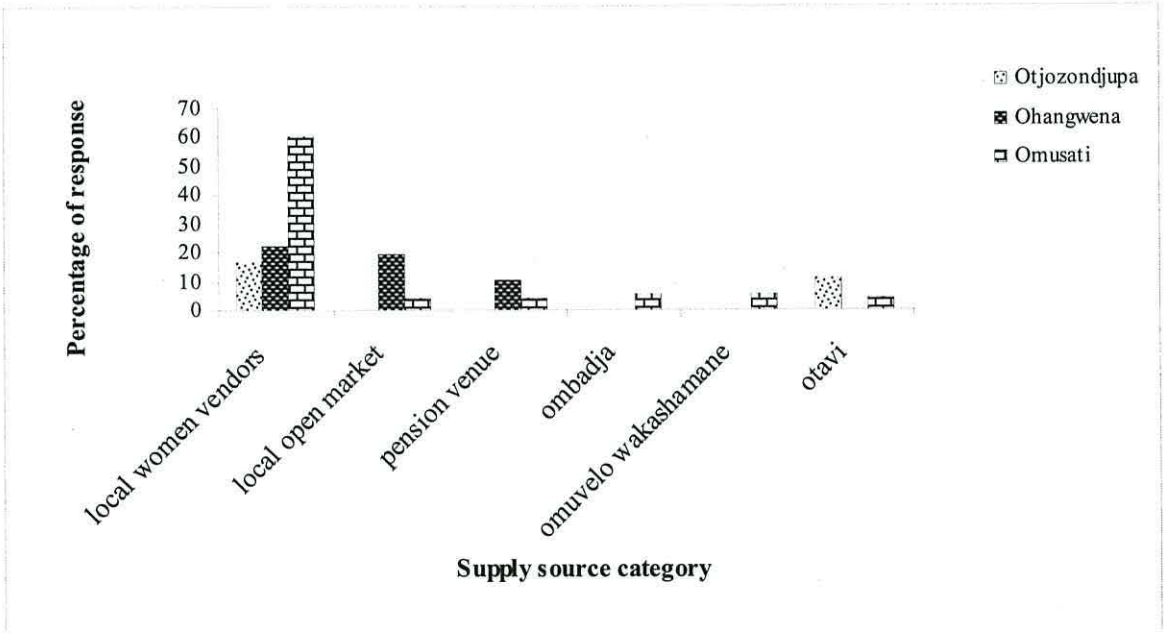


Figure 3.16 Percentage of response for *A. zebrina* flower products source of supply in Namibia.

The amount of flower products purchased by respondents varied between regions. The highest number of cakes and dried 'spinach' in kg purchased per household per season was recorded in Otjozondjupa, and the lowest in Ohangwena with no dried 'spinach' purchased in this region (Figure 3.17 and Figure 3.18, respectively). The mean number of cakes purchased per household per season in Otjozondjupa was 67±42 and 388±384 kg 'spinach' and in Ohangwena 9.20±3.40 cakes and no 'spinach' purchased in this region.

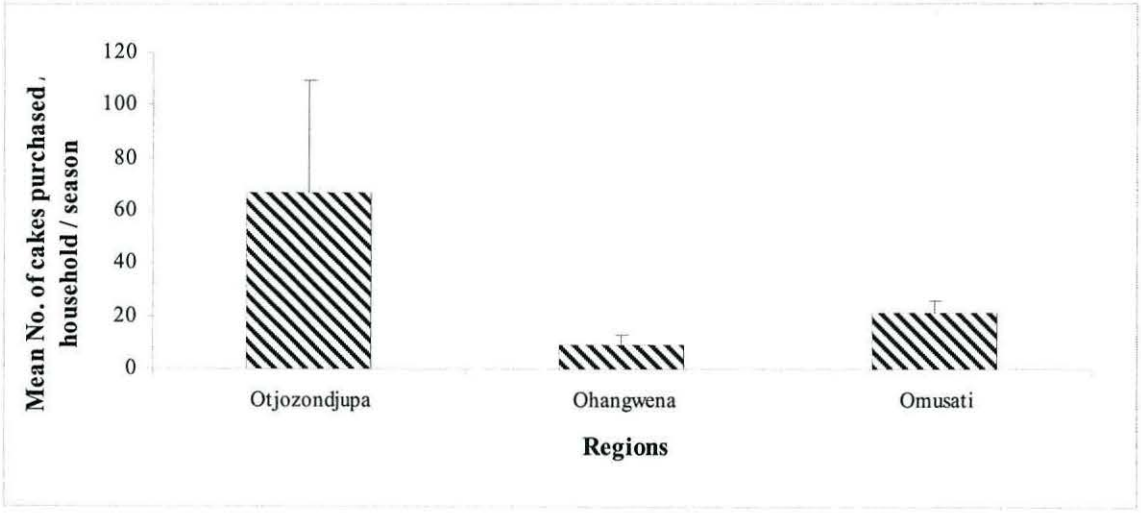


Figure 3.17 Average number of *A. zebrina* cakes purchased per household per season in Namibia

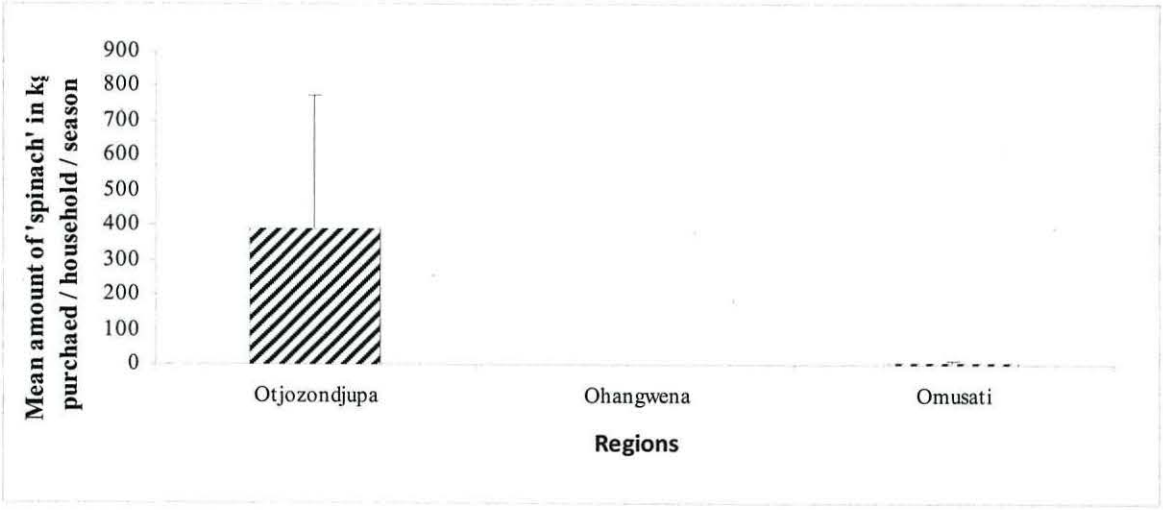


Figure 3.18 Average number of *A. zebrina* 'spinach' in kg purchased per household per season in Namibia

Although the majority of respondents in all the three regions (Otjozondjupa with 100% responses) did not know how the availability of processed *A. zebrina* flowers could be improved, suggestions such as planting more *A. zebrina* plants and the introduction of a harvesting permit were made by some respondents in Ohangwena and Omusati regions, while creation of markets for trading *A. zebrina* flower products was proposed by few respondents in Ohangwena region only (Table 3.10).

Table 3.10 Observed frequency of responses on how the availability of *A. zebrina* flower products could be improved in Namibia

	How *A. zebrina* flower products availability can be improved				
Region	**Do not know**	**Plant more *A. zebrina***	**Set up markets**	**Introduce harvest permit**	**N**
Otjozondjupa	18	0	0	0	18
Ohangwena	51	10	5	1	67
Omusati	67	4	0	1	72

The cost of processed *A. zebrina* flower cake as well as dried 'spinach' per kg was established. The results showed that there was a difference in the cost of a single cake as well as 1 kg of dried 'spinach' in all the three regions. The study also found that the cost of cake varied depending on its size and the area where the product was being sold. The mean cost ranged from N$1.85±0.16 per cake and N$1.10± 0.84 per kg 'spinach' in Ohangwena to N$3.18±0.25 per cake and N$26.47±1.91 per kg 'spinach' in Otjozondjupa as shown in figure 3.19 and figure 3.20, respectively (1N$ (Namibian Dollar) = 0.14US$, rate valid at 18/09/09).

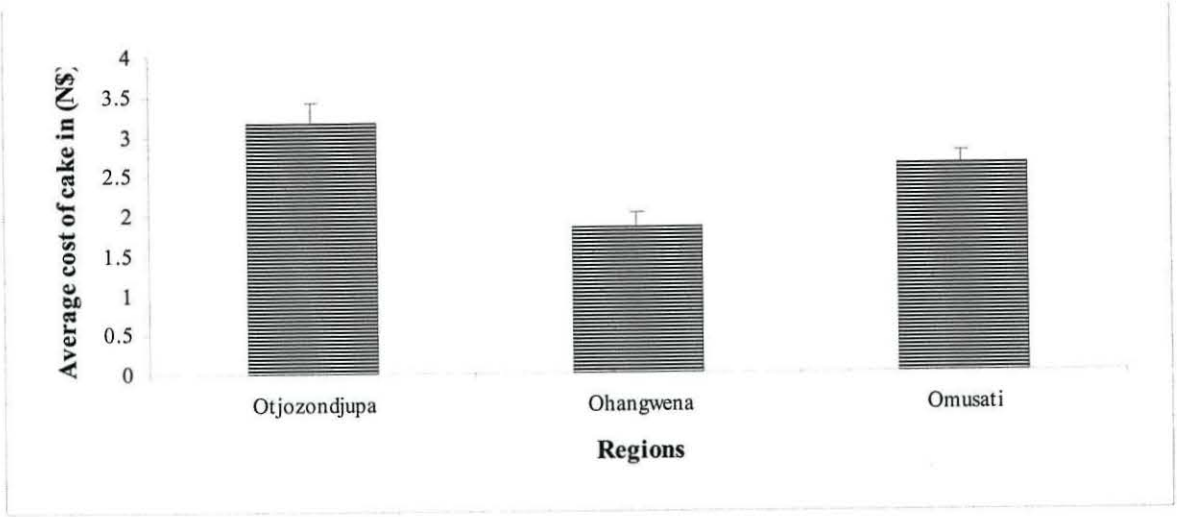


Figure 3.19 Average cost of *A. zebrina* flower cake in Otjozondjupa, Ohangwena and Omusati regions, Namibia

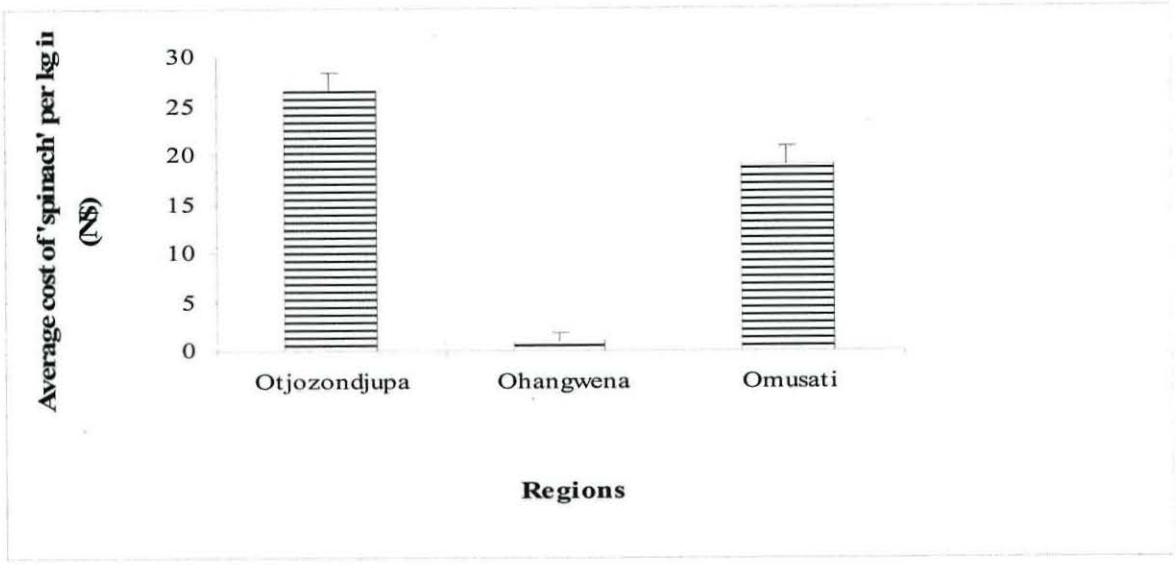


Figure 3.20 Average cost of *A. zebrina* flower 'spinach' per kg in Namibia

Despite better supply of *A. zebrina* flower products in Omusati (76.4%) followed by Otjozondjupa (55.6%) with least supply in Ohangwena (34.2%) (Table: 3.9), the price of flower cake as well as dried 'spinach' per kg was still high in Otjozondjupa and Omusati compared to Ohangwena region (Figure 3.19 and Figure 3.20, respectively).

3.4.1.7 Markets and income generation

The current study found that local buyers were regarded as the main buyers in all the three regions and the only buyers in Omusati region. In Otjozondjupa, other buyers included travellers and any other customers. Meanwhile, in Ohangwena region besides anyone, open market and school teachers were the other buyers identified (Figure 3.21).

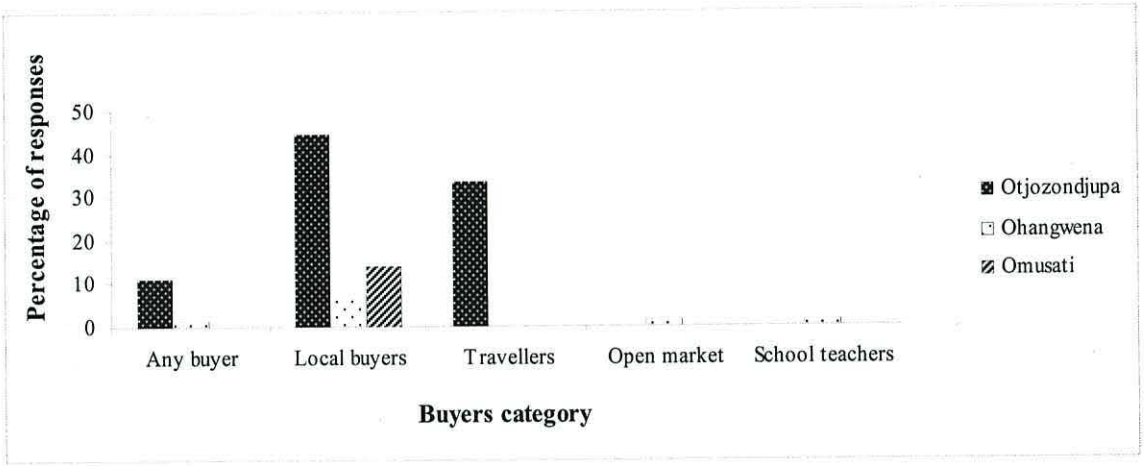


Figure 3.21 Percentage of response for buyers of *A. zebrina* flower products in Namibia

The number of cakes and the amount of dried 'spinach' in kg respondents sold varied considerably between regions. The highest number of cakes and the amount of dried 'spinach' sold per respondent was recorded in Otjozondjupa and the least in Ohangwena. The mean number of cakes sold in Otjozondjupa was 643±122 and 1369±1080kg 'spinach' compared to 7.30 ± 3.70 cakes and 0.08 ± 0.08 'spinach' sold in Ohangwena region (Figure 3.22 and Figure 3.23).

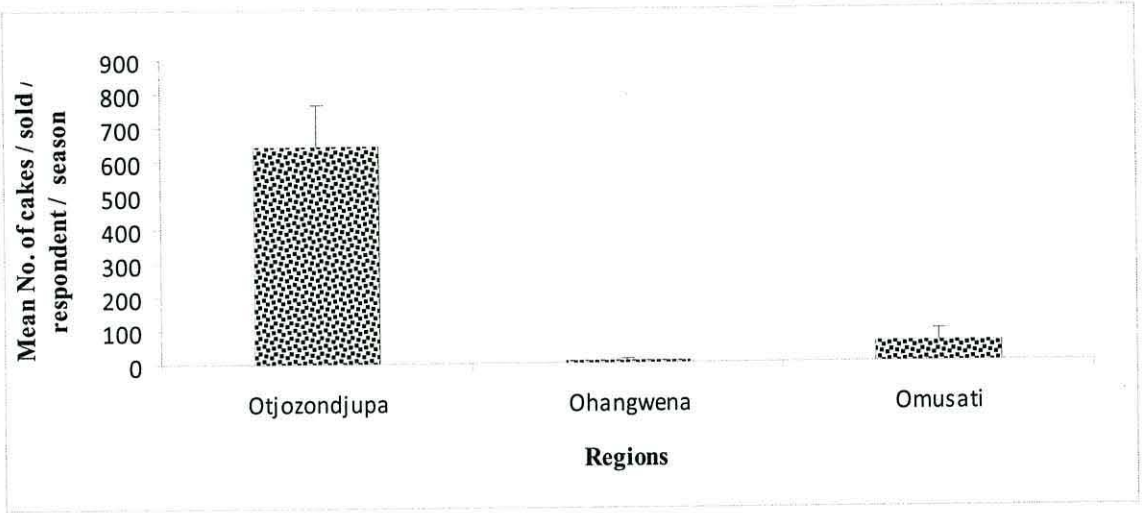


Figure 3.22 Average number of *A. zebrina* cakes sold per respondent per season in Namibia

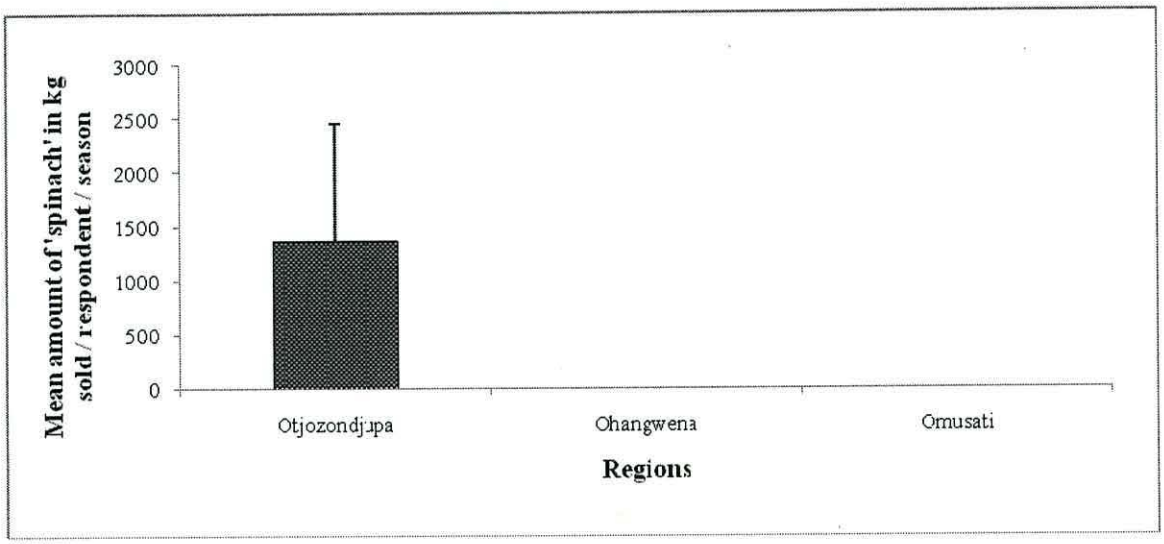


Figure 3.23 Average amount of *A. zebrina* 'spinach' in kg sold per respondent per season in Namibia

In response to the question whether *A. zebrina* flower products contributed to the respondents' household income, 100% of the respondents in Otjozondjupa confirmed while in Ohangwena region only 17.9% did (Table 3.11).

Table 3.11 Observed Frequency of responses on whether *A. zebrina* flower products contributed to respondents' household income in Namibia

	Whether *A. zebrina* flower products contribute to respondents' household income		
Region	**Yes**	**No**	**N**
Otjozondjupa	18	0	18
Ohangwena	12	54	66
Omusati	28	44	72

3.4.1.8 *A. zebrina* as animal food resource

The study noted that *A. zebrina* is also a source of forage to animals such as goats, cattle, kudus, donkeys, sheep, elephants, horses, porcupines, rabbits, springboks, wildebeests and bushbucks. It was also unveiled that chicken, ostriches, dogs and pigs were noticed eating *A. zebrina* flower products, while birds and bees are regular visitors to *A. zebrina* plants (Table 3.12). Uncooked flowers were the most preferred feed type, followed by leaves and cooked flower products. According to respondents, goats seemed to feed more on *A. zebrina* compared to other organisms. The majority of respondents were aware of the use of the plant as source of animal feed. The level of knowledge was noted to be 77.8%, 31% and 10.4% at Otjozondjupa, Omusati and Ohangwena, respectively (Figure 3.24).

Table 3.12 Animal that feed on *A. zebrina* plant parts and its products in Namibia

Animals feed on *Aloe zebrina*	**Number of people reporting on plant part / products fed on by animals**											
	branches	*Cakes*	*cooked flowers*	roots	raw flowers	Fruits	Leaves	*nectar*	panicles	whole plant	**Total**	% response
Bees	0	0	0	0	0	0	0	1	0	0	**1**	0.7
Bird	0	0	0	0	4	0	1	3	0	0	**8**	5.7
Cattle	0	0	0	0	5	0	12	0	1	0	**18**	12.7
Chicken	0	3	7	0	5	0	3	0	0	0	**18**	12.7
Dog	0	0	1	0	0	0	0	0	0	0	**1**	0.7
Donkey	0	0	0	0	6	0	8	0	0	2	**16**	11.4
Elephant	1	0	0	0	1	0	1	0	0	0	**3**	2.1
Goat	0	2	3	0	17	1	13	0	0	0	**36**	25.5
Horse	0	1	0	0	1	0	0	0	0	0	**2**	1.4
Kudu	0	0	0	1	14	1	2	0	0	0	**18**	12.7
Ostrich	0	0	0	0	2	0	0	0	0	0	**2**	1.4
Pig	0	0	0	0	0	0	1	0	0	0	**1**	0.7
Porcupine	0	0	0	0	2	0	0	0	0	0	**2**	1.4
Rabbit	1	0	0	0	1	0	0	0	0	0	**2**	1.4
Sheep	0	0	0	0	3	1	6	0	0	0	**10**	7.1
Springbok	0	0	0	0	1	0	0	0	0	0	**1**	0.7
Wildebeest	0	0	0	0	1	0	0	0	0	0	**1**	0.7
Bushbuck	1	0	0	0	0	0	0	0	0	0	**1**	0.7
Total	**3**	**6**	**11**	**1**	**63**	**3**	**47**	**4**	**1**	**2**	***141***	100
% response	2.1	4.3	7.8	0.7	44.7	2.1	33.3	2.8	0.7	1.4	**100**	

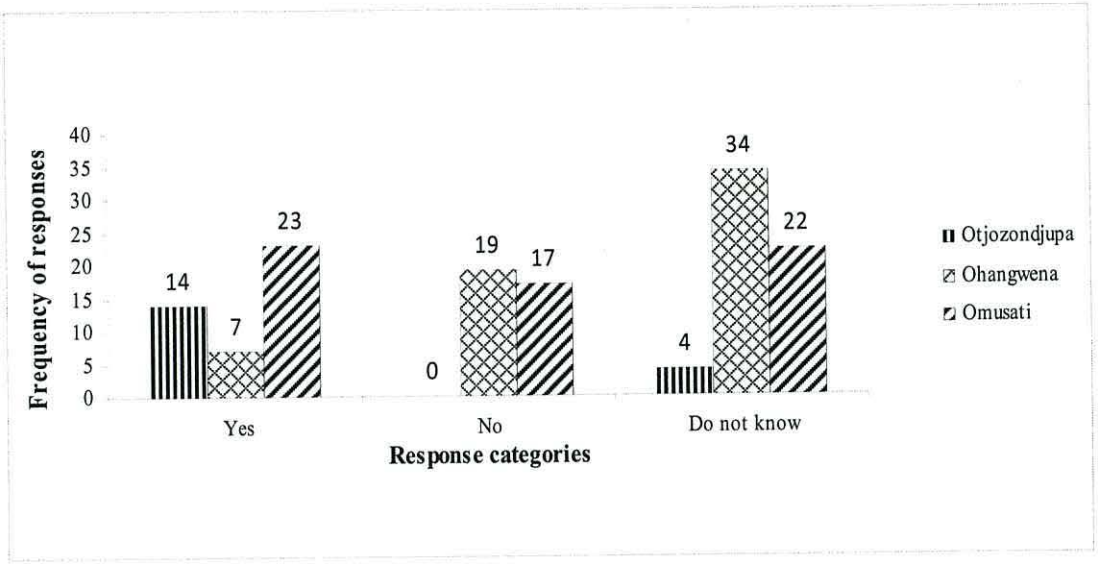


Figure 3.24 Frequency of response for knowledge on *A. zebrina* as animal feed in Namibia

3.4.1.9 Medicinal value

Knowledge about the medicinal value of *A. zebrina* was limited in all three regions. Only 11%, 31% and 34% of respondents in Otjozondjupa, Omusati and Ohangwena respectively considered the species to have medicinal value. For those who considered the species to have medicinal value, 69% considered roots to be the most frequently used, while 31% considered leaves to have potential for medicine. The disease or conditions treated included expelling placenta and treating eye disorders (Table 3.13).

Table 3.13 *A. zebrina* plant parts used for medicinal purposes, diseases or conditions treated and methods used in treatment in Otjozondjupa, Ohangwena and Omusati regions, Namibia

	Aloe zebrina plant part used for medicinal purposes				
Diseases /conditions treated	**leaves**	**roots**	**Total**	**% responses**	**Method of preparation**
cattle eyes	1	0	**1**	1.8	
human ears	2	0	**2**	3.6	
human eyes	6	5	**11**	20.0	Squeeze leaf juice directly into a sick person eyes
chicken cough	1	0	**1**	1.8	Put crushed leaves into chicken's water trough
human cough	1	3	**4**	7.3	Boil roots or just crush them and mix them with warm water, then drink the mixture as medicine
expel placenta	1	20	**21**	38.2	Pounded / crushed leaves or usually roots mix them with warm water and give to a human being, a cow or goat that has just delivered (but the placenta is not expelled)
skin rashes	1	2	**3**	5.5	Crush the roots, mix them with water, then apply the mixture over the skin
skin burns	1	0	**1**	1.8	
stomach-ache	1	0	**1**	1.8	
Ticks	1	0	**1**	1.8	
Wounds	1	0	**1**	1.8	
improve appetite	0	1	**1**	1.8	
Malaria	0	3	**3**	5.5	Pound roots, boil them for a while, give cooled water mixture to a Malaria patient to drink
stomach disorder	0	1	**1**	1.8	Pound roots, mix them with warm water then *'nyongama'*
Oshitwa	0	1	**1**	1.8	
Onhadi	0	1	**1**	1.8	Pound roots, mix them with warm water, give to a women who just deliver to ease the pain
Endjadja	0	1	**1**	1.8	
Total	**17**	**38**	**55**	**100**	
% responses	30.91	69.10	**100**		

3.4.1.10 Nutritional value

Knowledge about the nutritional value of *A. zebrina* flower products was investigated. Respondents felt that there should be some nutritional value in the product such as energy, vitamins and other nutrients (Figure 3.25). However, there was no further specification since the nutritional status of this food was unknown.

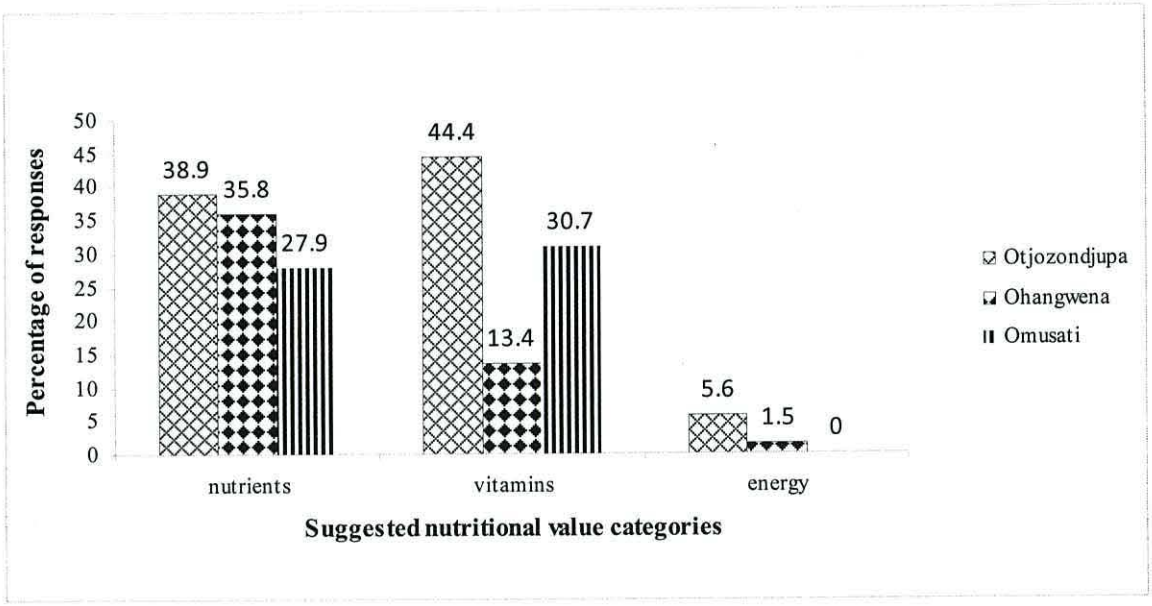


Figure 3.25 Percentage of responses on suggested nutritional value of *A. zebrina* flower products in Otjozondjupa, Ohangwena and Omusati regions, Namibia

3.4.1.11 Gender role

The current study found that harvesting of *A. zebrina* flowers was carried out by both women and men in all the three regions (Figure 3.26). Result presented in Figure 3.26 shows that in Otjozondjupa and Omusati regions, there was no major gender divide as far as harvesting of *A. zebrina* flowers was concerned (any sex, 72.2%; women only, 27.7%; N = 18) and (any sex 48.7%; women only 45.8%; N = 66) respectively. However, in Ohangwena gender seemed to be an issue when it comes to harvesting of *A. zebrina* flowers (any sex, 35.8% and women only, 62.7%; N = 71).

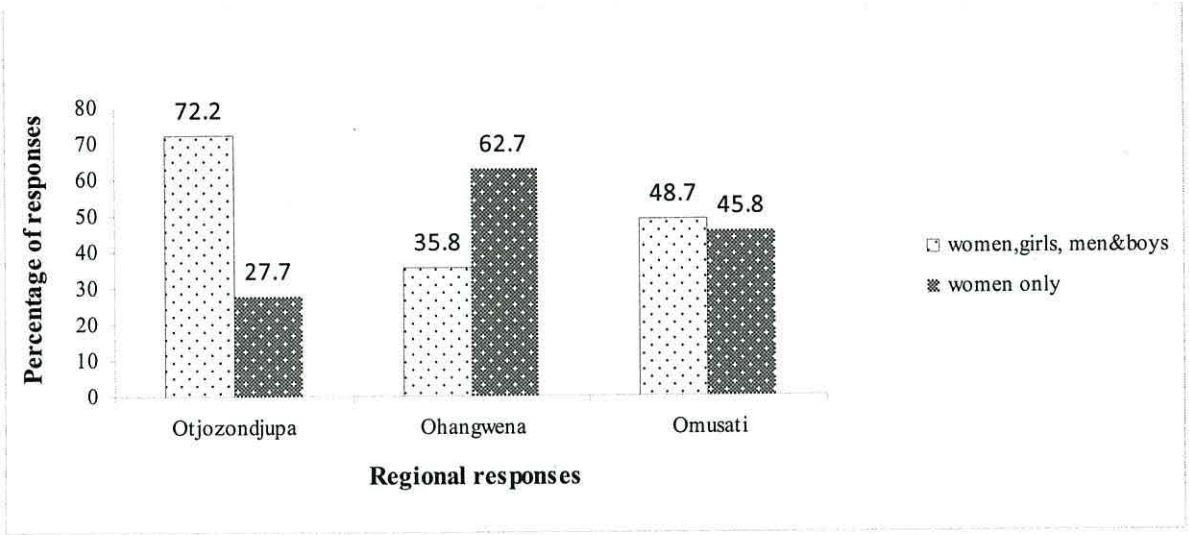


Figure 3.26 Percentage of response on *A. zebrina* flowers harvesting in Namibia

3.4.1.12 Land tenure

3.4.1.12.1 Location

This study also investigated the location where *A. zebrina* flowers were collected in all the three regions (Figure 3.27). There was a difference between regions in the number of locations where flowers were collected from. Omusati region had a diverse number of locations (cultivated area / land within vicinity locally known as *mepya*, 1.4%; uncultivated area within vicinity locally known as *mekove*, 43%; outside the fence near the vicinity locally known as *konima yongubu*, 8.3%; outside the fence far from the vicinity, 1.4% and anywhere, 40.3% ; N = 68), followed by Ohangwena region (cultivated area within vicinity, 19.4% ; uncultivated area within vicinity, 14.9% ; outside the fence near the vicinity, 10.4% and anywhere, 53.7%; N = 66) and the least was Otjozondjupa (uncultivated area within vicinity, 94.4% and outside the fence near the vicinity, 5.6%; N = 18).

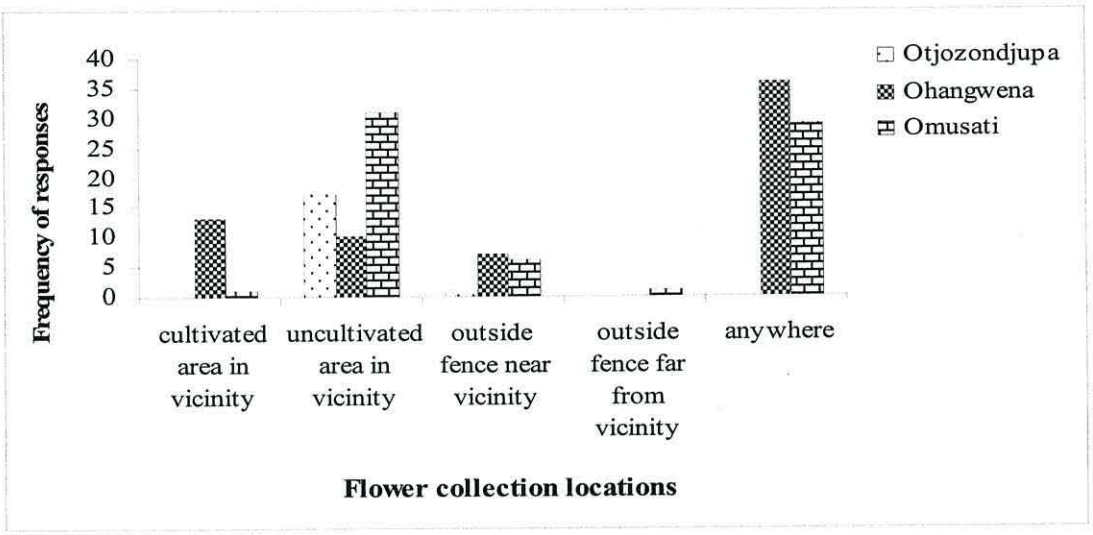


Figure 3.27 Frequency of responses on location where *A. zebrina* flowers are harvested in Namibia

3.4.1.12.2 Land Ownership

During the present study, three land ownership categories where *A. zebrina* flowers being harvested were identified as: land owned by commercial farmers; communal land and respondents own land (Figure 3.28). Results in Figure 3.28 show that respondents from Omusati region, collected flowers from all the three land ownership categories (communal 81.9%, commercial 6.9% and own land 5.6%), in Ohangwena flowers were only collected from communal land (88.1%) and own land (3.0%) while in Otjozondjupa region, 94.5% of respondents harvested flowers from commercial farmers' land.

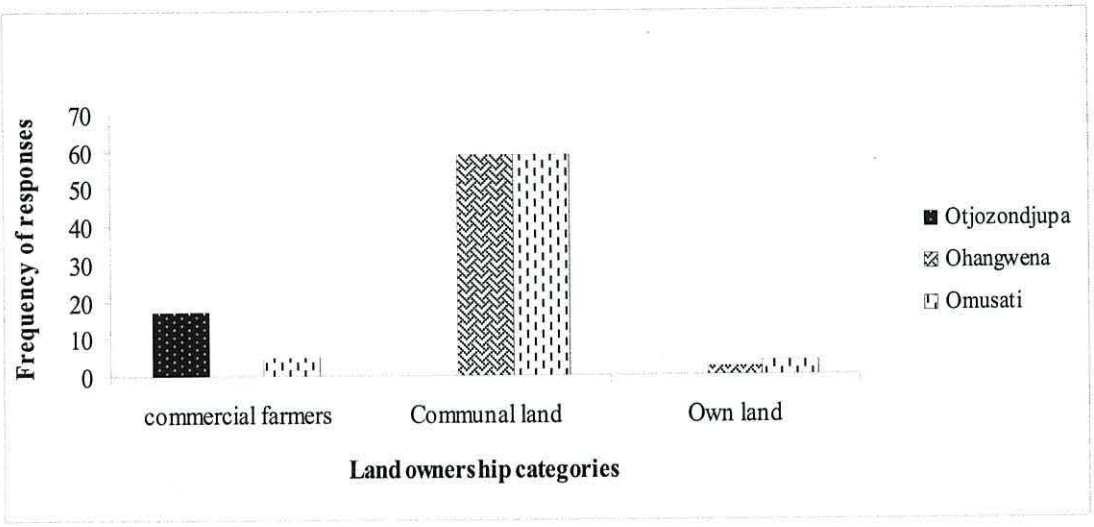


Figure 3.28 Frequency of responses on land ownership categories where *A. zebrina* flowers are harvested in Namibia

3.4.1.13 Domestication

Results on assessing indigenous knowledge whether *A. zebrina* can be domesticated showed that more than 80% of respondents in all three regions indicated that the species can be domesticated, Ohangwena (98.4%, N = 63), Omusati (93%, N = 67) and Otjozondjupa (82.4%, N = 17). The study also found the reasons why participants thought *A. zebrina* be domesticated, and the following reasons were identified; grows easily, wildling possible, requires less maintenance and drought tolerant. Reasons why respondents felt that the species can grow easily was reported in all three regions; Ohangwena (100%, N = 58); Omusati (75.8%, N = 62) and Otjozondjupa (37.5%, N = 8). Possibility of wildling was only recorded in Omusati (24.2%, N = 62) while less maintenance and drought tolerant (37.5% and 25%, N = 8) respectively, were only mentioned in Otjozondjupa region (Figure 3.29).

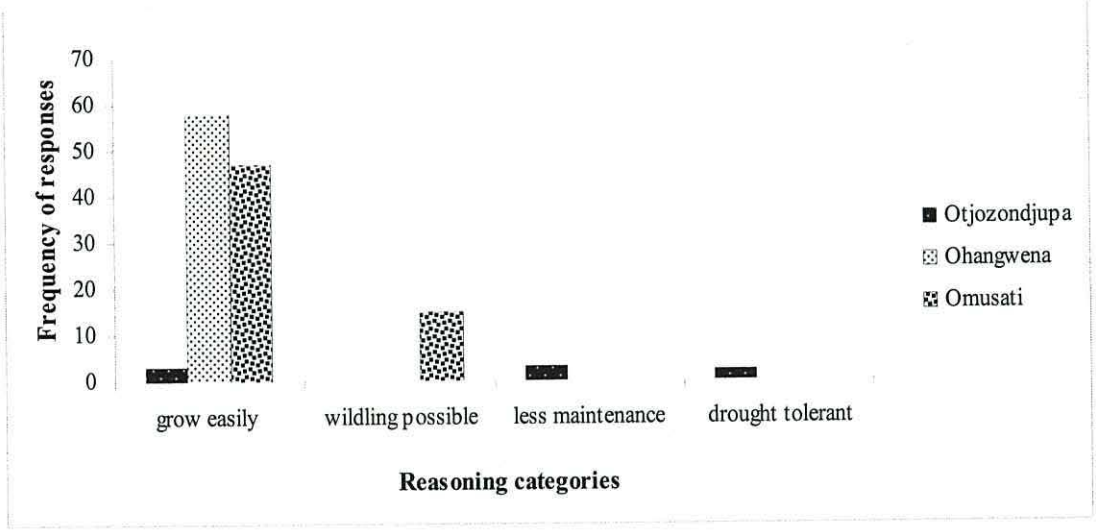


Figure 3.29 Frequency of responses on reasoning for domestication of *A. zebrina* species in Namibia

3.4.1.14 Propagation

According to some respondents, propagation of *A. zebrina* was attempted in all three study regions. The highest percentage of attempts to propagate the species was recorded in Ohangwena region (65.7%, N = 67), and the lowest record was in Omusati region (18.1%, N = 72) (Table 3.14).

Table 3.14 Observed frequency of responses on propagation attempts of *A. zebrina* species in Namibia

Region	Yes	No
Otjozondjupa	27.8 (18)	72.2
Ohangwena	65.7 (67)	34.3
Omusati	18 (72)	82

Participants suggested methods that could be used in propagating *A. zebrina* species namely; transplanting of young wildlings of *A. zebrina* and all wildings regardless of age. The latter was only reported in Ohangwena region (Figure 3.30).

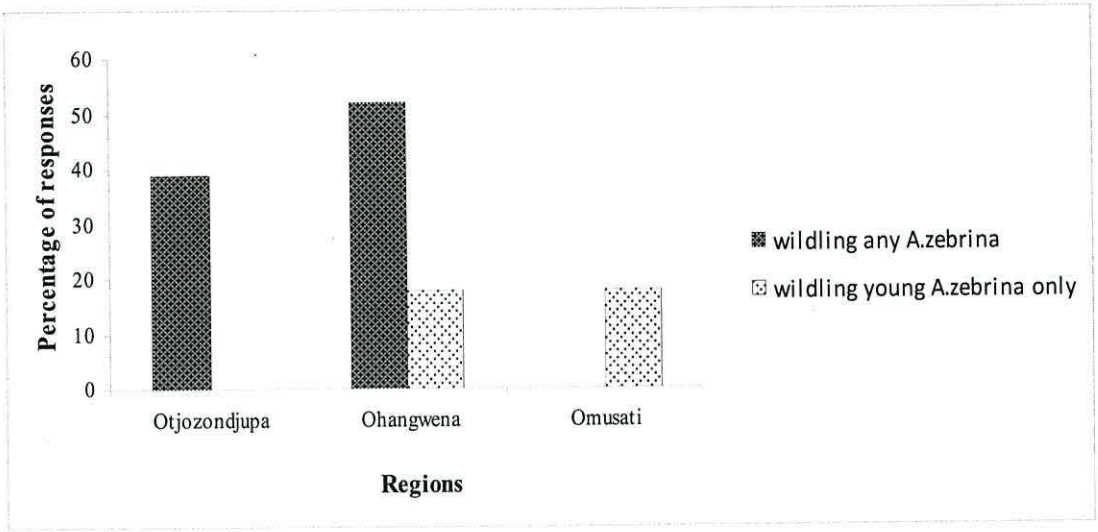


Figure 3.30 Frequency of responses on proposed methods for propagating *A. zebrina* species in Namibia

3.4.1.15 Management

Assessment on the practice of uprooting *A. zebrina* plants for either transplanting or other purposes was carried out as shown in Figure 3.31. More than 88.9% of respondents from all three regions responded by saying that they never uprooted the species.

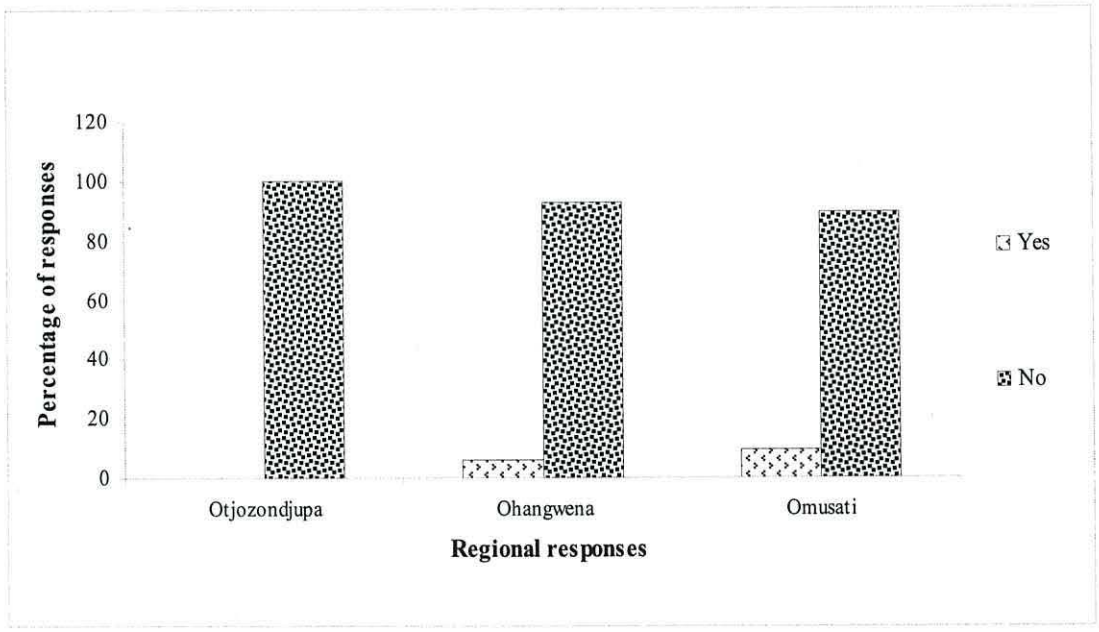


Figure 3.31 Percent of responses on uprooting of *A. zebrina* plants in Namibia

Participants were also asked about types of management aspects they considered important for *A. zebrina*. The following management aspects were suggested; fencing, avoid damaging plant, less watering in dry season, weeding, avoid panicle breaking, avoid root cutting and no watering in rain season (Figure 3.32) .
Findings revealed that respondents from Ohangwena region gave the highest number of proposed management aspects compared to Omusati and Otjozondjupa regions.

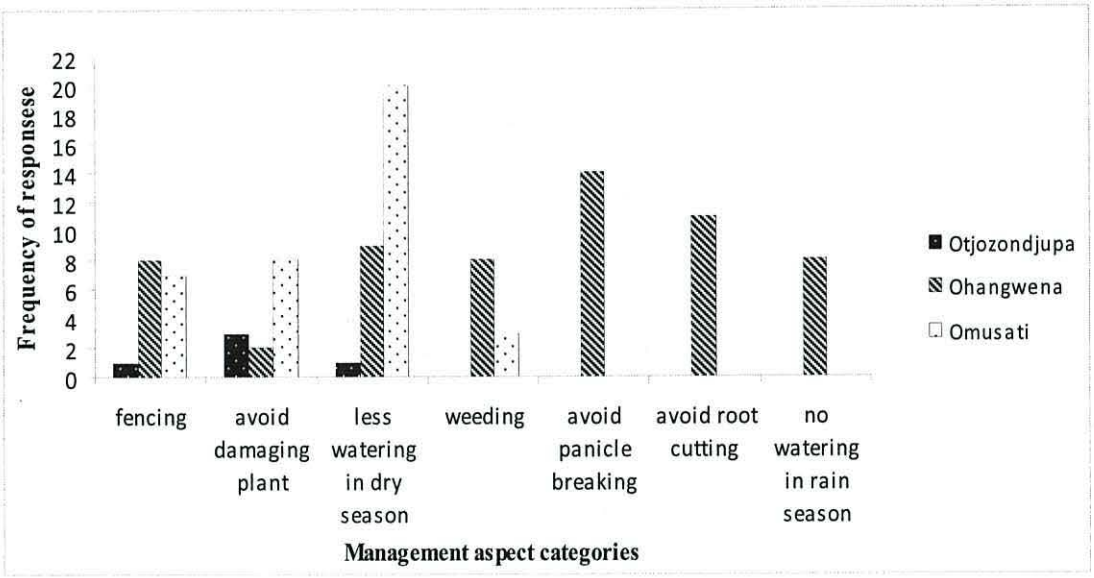


Figure 3.32 Frequency of responses on proposed management aspects important for *A. zebrina* species in Namibia

3.4.2 Trial on method of low cost propagation at Ogongo Agricultural College Namibia

The results of the propagation trial indicated that *A. zebrina* can easily be propagated from seeds.

Seed emergence was observed starting from 5 – 7 days after sowing and 100% germination was observed between 3-4 weeks after sowing. All seeds sown in October 2006 germinated and grew into adult plants one year after sowing. The number of leaves per plant ranged between 7 and 11 (Figure 3.33). All seeds sown in February 2007 germinated but one died at the end of October 2007, nine months after

sowing (Figure 3.34). They also grew fully and the number of leaves per plant ranged between 5 and 7.

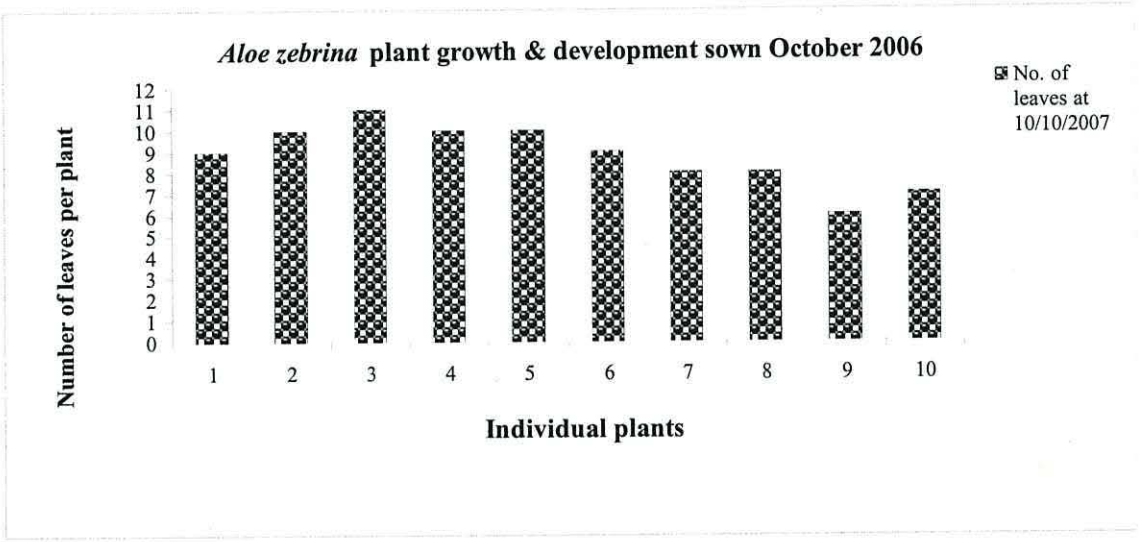


Figure 3.33 Number of leaves per plant for growth and development assessment

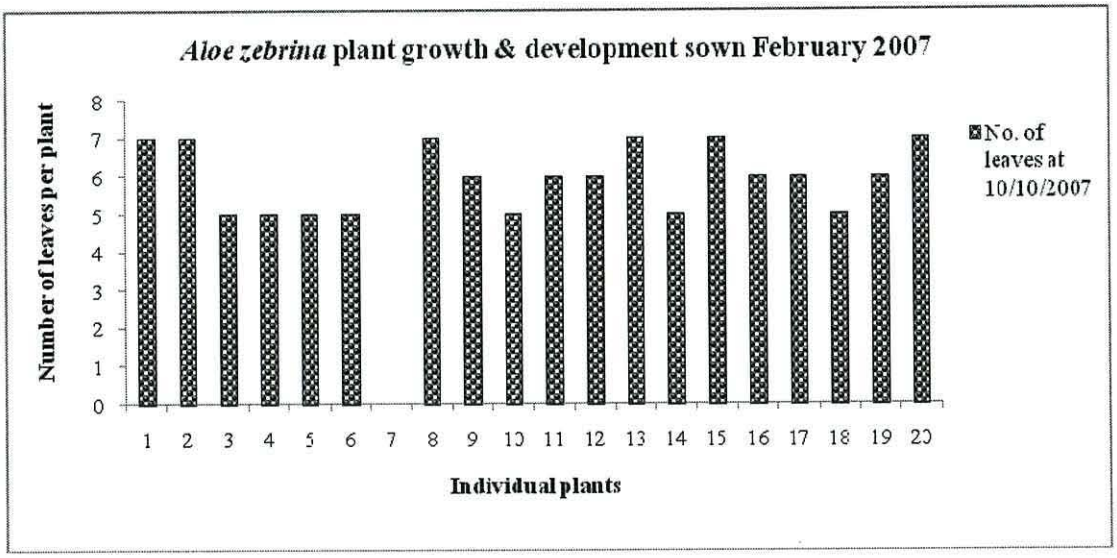


Figure 3.34 Number of leaves per plant for growth and development assessment

3.5 Discussion

3.5.1 Awareness on species existence and status

Respondents in all the three study sites were very well aware of the existence of *A. zebrina* with the majority of them confirming the occurrence of the species in their villages. Indigenous knowledge assessment on whether the species has more than one variety, indicated that the majority of respondents in Otjozondjupa and Omusati regions claimed the species has only one variety. But in Ohangwena region participants had mixed reactions. This is probably due to clinal variation of the species as reported by Jeppe (1969) and Van Wyk (1996). Results of a further assessment of whether the species have male and female reproductive organs on separate plants or hermaphrodite (have male and female reproductive organs on the same plant), showed that majority of respondents from Otjozondjupa were very well aware that *A. zebrina* is hermaphrodite. This is in agreement with the findings reported by Reynolds (2004) that *A. zebrina* is a hermaphrodite plant. The majority of respondents in Ohangwena region, however, did not know whether *A. zebrina* is hermaphrodite or not. This may suggest that respondents in Ohangwena region had limited indigenous knowledge about *A. zebrina* compared to those in Omusati and Otjozondjupa regions.

The results of local knowledge assessment whether respondents noticed any change in *A. zebrina* species abundance in the past 20 years (1987-2007), showed that a decrease in species abundance was experienced by respondents in Omusati and Otjozondjupa regions, while respondents in Ohangwena region reported a significant increase in the species abundance in their region. This is in line with findings reported in Chapter 4 of this thesis, whereby the highest number of *A. zebrina* plants with highest number of regeneration from seeds was recorded in Ohangwena region. In addition, perhaps because of overexploitation of the species in Otjozondjupa and Omusati regions compared to Ohangwena region, the species is in decline in these two regions.

3.5.2 Harvesting and utilization

Involvement of the majority of respondents (>80%) in harvesting of *A. zebrina* flowers was confirmed in all the three study regions, which is an indication of the full utilisation of the species in Namibia. The study also unveiled that a harvesting permit for *A. zebrina* flowers was only a requirement in Otjozondjupa region. This is probably because the study site Otavi (based in Otjozondjupa region) is a commercial farm area and *A. zebrina* flower collectors needed to obtain permit to harvest flowers from the private commercial farm owners.

Harvesting time of *A. zebrina* in Otjozondjupa (May – September) was different from Ohangwena (January – April) because of the difference in the flowering season between the two regions. However, an overlap in the species harvesting time reported in Omusati (February – September) is probably due to the fact that respondents in Omusati region harvested the species from Omusati as well as in the neighbouring southern Angola during February – April, which is the flowering season for *A. zebrina* in these areas as documented by PROTA (2008), and they also travelled to Otjozondjupa region for harvesting flowers, when the species is in flowering season from May to September.

The present study also found three main reasons why *A. zebrina* flowers were harvested by the respondents. These included: for domestic consumption only, for sale only, and for both domestic consumption & sale. The study also discovered differences in priorities that the *A. zebrina* flower harvesters had when harvesting flowers. Respondents in Otjozondjupa region reported harvesting *A. zebrina* flowers for both home consumption and sale as their main priority for harvesting the species. While respondents in Ohangwena region stressed that harvesting the species for home consumption was their main priority, and none of them harvested the species for sale. This may be due to the fact that there was less supply of flower products in this region than in the other two regions. Meanwhile in Omusati region, although respondents indicated harvesting the species for home consumption as their main priority, they also indicated harvesting the species for both home consumption and sale as their second priority. This might be because they have a better access to the

resource in the first place from their region, secondly from Ombadja (southern Angola) and thirdly from Otjozondjupa region.

The current study found two products obtained from *A. zebrina* flowers: cake and 'spinach' locally called *oshinyanekela* in Oshiwambo language. Processing methods of the two products were also described. The number of cakes produced from a single *A. zebrina* plant indicated that the amount of flowers produced from a single plant was not enough to make one full cake. This means one needed to collect flower from more than one plant to make a cake. However, there was a slightly differences between regions on how much flowers could be collected from a single plant. The consumption of cake and spinach per household indicated that Otjozondjupa had the highest number of both cakes and dried 'spinach' consumed per household compared to Ohangwena and Omusati regions. This is probably because the species is a more popular and desirable delicacy in Otjozondjupa region than in other regions.

The results on the taste of *A. zebrina* flower products indicated that respondents favoured the natural sweet taste of the flower products without the addition of any other ingredient (>90% respondents). Although some ingredients that could be added to cooked flower products were reported, respondents stressed that addition of other ingredients could not improve the taste of *A. zebrina* flower products very much.

The majority of respondents purchased flower products for consumption in all the three regions, with the highest amount of cakes and spinach purchased per household in Otjozondjupa region because of the popularity and desirability of flower products as a delicacy in this region. It is also interesting to note that the cost of flower cake and dried 'spinach' was the highest in Otjozondjupa region. This may be explained by Say's Law which states that supply creates its own demand (Daly, 2004). The results of the investigation into marketing and generation of income from *A. zebrina* flower products also indicated that the flower products were only traded locally, and the amount traded varied considerably between regions. The highest amount sold (both cakes and dried 'spinach') per household per season was recorded in

Otjozondjupa and the least is in Ohangwena. Based on these findings, most households in Otjozondjupa were selling flower products for income generation.

The current study has unveiled that many people seemed to be aware about the use of *A. zebrina* as a source of animal feed, with the highest level of awareness noted in Otjozondjupa and the least in Ohangwena. Chickens, dogs, pigs and ostriches were also noticed eating processed flower products, while birds and bees were also observed visiting *A. zebrina* flowers regularly as reported by Reynolds (2004). During this study it was found that goats seemed to feed more on *A. zebrina* plants compared to other organisms.

Investigation into awareness on the importance of *A. zebrina* plant for medicinal value indicated that there was limited awareness in all study regions reflected by poor regional responses. Results showed that *A. zebrina* roots were the most important part of the plant considered for medicinal value, followed by the leaves. One of the conditions treated using *A. zebrina* included expelling the placenta during labour for human beings, cows and goats. Similar use of other *Aloe* spp. (*Aloe cooperi* Baker and *Aloe ecklonis* Salm-Dyk) in postnatal care has been documented by Grace *et al.* (2008) in Southern Africa. The second condition treated using *A. zebrina* was treatment of eye disorders as reported by Leffers (2003). Similar application of the leaf exudates of *Aloe ferox* as an eye drop for treating eye ailments in Southern Africa was reported by Grace *et al.* (2008).

The majority of respondents harvested flowers from communal lands (82%). In Otjozondjupa region, however, 95% of the respondents harvested flowers from privately owned commercial farms in the vicinity of Otavi mountain, an area referred to as the "Golden Triangle" as documented by Heyns (2008).

Results of the investigation into gender role in harvesting *A. zebrina* flowers indicated that both women and men were involved in harvesting flowers in all the study regions. However, in Ohangwena region gender seems to be an issue when it comes to harvesting of *A. zebrina* flowers, with 63% of respondents indicating that harvesting of *A. zebrina* flowers was women's role. The study also found that local

women vendors were the major suppliers of *A. zebrina* flower products in all three study regions. This is an indication that women played a major role in the supply of *A. zebrina* flower products. This is in line with the general belief that women possessed more knowledge about wild foods than men and the reason why they are primarily responsible for collection and preparation of wild foods in many rural communities (Harris and Salisu, 2003).

Results of the assessment of indigenous knowledge whether *A. zebrina* could be domesticated showed that >80% of respondents in all the study regions indicated that the species could be domesticated easily. Similar suggestion was made by Rothmann (2004). The use of wildlings was suggested as one of the ways for propagating *A. zebrina* and was reported to be practised widely in Ohangwena region. Although during this study *A. zebrina* seeds emergence was observed 5-7 days after sowing and 100% germination was observed 3-4 weeks as reported by Rothmann (2004) and Plantzone (2009). The present study, therefore, showed that *A. zebrina* is easily propagated from seeds and this may offer an alternative to uprooting of wildlings and arrest further erosion of the germplasm of this important resource in Namibia.

CHAPTER 4

ECOLOGY AND POPULATION STATUS OF *ALOE ZEBRINA* BAKER IN NAMIBIA

This chapter examines the ecology and population status of *A. zebrina* in Namibia. The chapter is presented in five sections subdivided into subsections, starting with a brief account of the state of knowledge and research objectives. In the third section procedures followed in data collection was presented, which is composed into soil characterizations, rainfall and temperature, population status, regeneration, associated plant species as well as explanation on how data were processed and analyzed. The fourth section presents the results of the current study. Meanwhile the last section discussed the findings based on the ecological conditions, population status, regeneration as well as *A. zebrina* associated plant species.

4.1 Background

The Population status of a plant species is determined based on an assessment of the population structure in terms of size class distribution of the species. Knowledge of the structure of the population of any species is of considerable importance for the management of the species because it provides knowledge on the amount of resource available, its distribution, its characteristics and changes occurring in the population, all of which provide a picture of the future prospects of the resource and how it can be managed sustainably (Mwang'ingo, 2002).

The existence of a species in a plant community largely depends on its regeneration status under varied environmental conditions. Regeneration is, thus, a critical phase of plant community management, simply because it maintains desired species composition and stocking after disturbances. The regeneration status of a species in a community can be assessed from the population dynamics of seedlings in the plant community. Several authors have assessed the regeneration status of tree species based on the age and diameter structure of their population. A population structure characterized by the presence of a sufficient number of seedlings depicts satisfactory

regeneration behaviour, while an inadequate number of seedlings in a plant community indicate poor regeneration. Moreover, successful regeneration of wild plants depends on its ability to produce large number of seedlings and their ability to survive and grow. However, the presence of a sufficient number of seedlings is greatly influenced by the interaction of biotic and abiotic environmental factors, whereby the intensity, magnitude and frequency of disturbances also determine the structure and composition of plant communities. Disturbances can have a negative impact, disrupting the climax and making it unstable, but they also have a positive impact by creating favourable niches for regeneration (Duchok, *et al.* 2005).

In Namibia, *A. zebrina* is found from the Grootfontein area downwards to Windhoek (the capital city of Namibia) and then east, north and across the Kalahari (Rothmann, 2004). However, the population status and regeneration of *A. zebrina* in these areas are not known. Although several studies have reported that the species is mostly found growing under bushes (Rothmann, 2004; Leffers, 2003; Von Koenen, 2001; Jankowitz, 1975), the plant species specifically associated with *A. zebrina* are not much explored. In this study the population and regeneration status of the species were assessed in three regions of Namibia.

According to MET (2010) nine major types of soils in north-central Namibia, which are silt, sodic sands, deep Kalahari sands, loams and clays, loams, clayey sodic sands, sands and loams, clayey sands and dolomite sands, are largely dominated by sands and clays which have been reworked and mixed by the action of water and wind. Their potential for crop cultivation is low in most areas for several reasons: poor water-holding capacity, low nutrient content, high salt content and hard layers of clay below the surface. Most plants characteristically grow on certain types of soils, so those in the eastern and western Kalahari sands are quite different from those in the Cuvelai and other landscapes. The semi-arid climate is characterized by rain that varies greatly in amount and timing. Almost all of the rain falls during the summer months (roughly November to April), and over two-thirds of it falls in January, February and March. That means there is a long dry season starting from roughly May to October. Eastern areas receive higher and more reliable rainfalls than the west, but much of the region is too dry for crop production. Rainfalls over the past 20

years have been generally lower than during the 1970s which, in turn, were wetter than the 1960s. The high degree of variation in rainfall means farming is a risky business. Good yields may be had in some years, but crops often fail as a result of inadequate or badly timed rainfalls. High evaporation rates and temperatures aggravate the effects of limited rainfall (MET, 2010). Therefore, soil samples and climatic data were also collected in order to characterize the site conditions of the three regions where *A. zebrina* grows.

4.2 Objectives

The present research was carried out with the aim of gathering information on the soil and climate of the study areas to characterize the site conditions of *A. zebrina* populations and assess the population status, natural regeneration as well as associated plants of *A. zebrina*. Specific objectives of the current study were;

a) To analyze and compare ecological conditions of Ohangwena, Omusati and Otjozondjupa regions where *A. zebrina* occurs, and

b) To assess the population status, natural regeneration and associated plant species of *A. zebrina*.

In order to address these objectives, the following hypotheses were formulated.

i. There is no ecological difference between the three study sites where *A. zebrina* occurs;
ii. There is no difference in population structure and regeneration status of *A. zebrina* between the three study regions;
iii. There is no difference in morphological characteristics of *A. zebrina* between the three study regions;
iv. There is no variation in the number and type of associated plants of *A. zebrina* between the three study regions.

4.3 Materials and methods

4.3.1 Data collection

4.3.1.1 Soil characterization

Soil properties were studied by collecting soil samples from the three regions. Soil samples were collected from Otavi Municipality plots and Shenga farm in Otjozondjupa region. In the second region (Ohangwena), sampling areas were Omhito ya Nanime, Omulamba and Onghwiyu villages. In the third region (Omusati) the sampling areas were Onambome, Ondudu and Omatwadiva villages. This made a total of 8 soil study areas; 2 from Otjozondjupa region and 3 from each of Ohangwena and Omusati regions.

In Otjozondjupa region, five soil sampling plots of 10 m x 10 m were laid out in each of the two study areas. In the Ohangwena and Omusati regions, three plots per study area were laid out. This gave a total of 28 plots for soil sampling. Soil samples were collected from two random locations within each plot. At each random location a trench was dug to expose the soil horizons. Two samples per trench (one at a depth of 0-25 cm and the second at a depth of 25-50 cm below the organic layer) or four samples per plot were taken (two of 0-25 cm depth and two of 25-50 cm depth). This gave a total of 112 soil samples (*i.e.* four soil samples per plot x 28 plots = 112 samples) for the whole study. Due to limited soil analysis consumables, the number of soil samples was reduced by bulking the two soil samples from each soil depth to produce one composite sample per soil depth. Thus, the total number of soil samples for the whole study was reduced from 112 to 56 soil samples.

During collection, samples were placed in sealed - labeled plastic bags. These were sent to the soil laboratory of Ministry of Agriculture, Water and Forestry in Windhoek, Namibia for analysis. The following soil properties were analyzed: pH, organic matter (%), nitrogen (%), phosphorus (mg kg^{-1}), potassium (mg kg^{-1}), calcium (mg kg^{-1}), magnesium (mg kg^{-1}), sodium (mg kg^{-1}), carbonate, texture (sand, clay and silt %) and electrical conductivity ($\mu S\ cm^{-1}$).

4.3.1.2 Rainfall and temperature

Climatic data for study regions were obtained from records of rainfall and temperature data at three weather stations, one in each region, Otavi station in Otjozondjupa region and Mahenene station in Omusati region. For Ohangwena region, however, records were collected from Ondangwa weather station (in Oshana region) which was the closest weather station to the study sites in Ohangwena region (25 km from the border of Ohangwena region).

4.3.1.3 Population status, regeneration and associated plant species

Population status of *Aloe zebrina* was studied in each study area by assessing the population structure, regeneration and associated plant species of trees and shrubs (Figure 4.1, 4.2 and 4.3). Population structure assessment was carried out in all the 28 plots in the eight study sites in the three regions described above (section 4.3.1.1).

Figure 4.1 *A. zebrina* population status assessments at Otavi, Otjozondjupa Region, Namibia

Figure 4.2 *A. zebrina* population status assessments at Onambome, Omusati Region, Namibia

Figure 4.3 *A. zebrina* leaf measurements at Ondudu, Omusati Region, Namibia

Within each study plot, all *A. zebrina* plants with six or more leaves were recorded as adults whereas plants with five or fewer leaves (including false leaves) were recorded as regeneration. This strategy was used considering reports by Rothmann (2004) and Plantzone (2009) that aloe seedlings must be left intact for one year and transplanting must be done at three to four true leaves. All *A. zebrina* plants within each plot were counted: both adults (>6 leaves) and regeneration (1-5 leaves). The number of leaves of each adult plant within each plot was counted. The height and diameter of five randomly sampled adult plants within each plot were measured. The source of regeneration (seed or root) was determined by digging up 1-5 leaved plants at random from each plot to see if regeneration was from seed or root.

During counting, adult plants were tallied into the following categories based on the number of leaves.

i. 6 – 10 leaves
ii. 11 – 15 leaves
iii. 16 – 20 leaves
iv. 21 – 25 leaves
v. 26 + leaves

Associated plant species (trees and shrubs) were identified and their frequencies recorded within each plot.

4.3.2 Data processing and analysis

Ecological conditions, population status and regeneration data were analyzed using Minitab 15 Software Package (Minitab Inc, USA). Pearson correlation analysis was used to establish relationships between parameters. In addition, Sorensen's index of similarity (Magurran, 1988) was used to determine similarity or difference in associated species between sites. The index was calculated using the following formula:

$Cs = 2j / (a + b)$, Where; j is the number of species found in both sites and a is the number of species in Site A with b is the number of species in Site B.

If the index is to equal 1 it shows complete similarity (that is where the two sets of species are identical) and 0 if the sites are dissimilar and have no species in common.

4.4 Results

4.4.1 Rainfall and temperature

4.4.1.1 Otjozondjupa region

Annual rainfall records between 1977 and 2006 from Otavi weather station (Figure 4.4) showed a mean annual rainfall of 472.1 mm for Otjozondjupa region. Mean annual temperature between 1952 and 1968 (Figure 4.5) was 20.4°C.

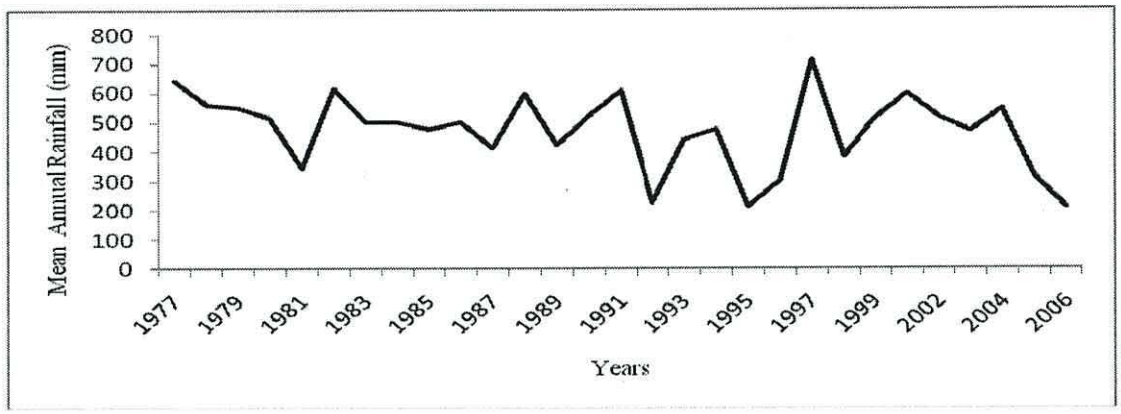


Figure 4.4 Average annual Rainfall (mm) recorded at Otavi station in Otjozondjupa region, Namibia.

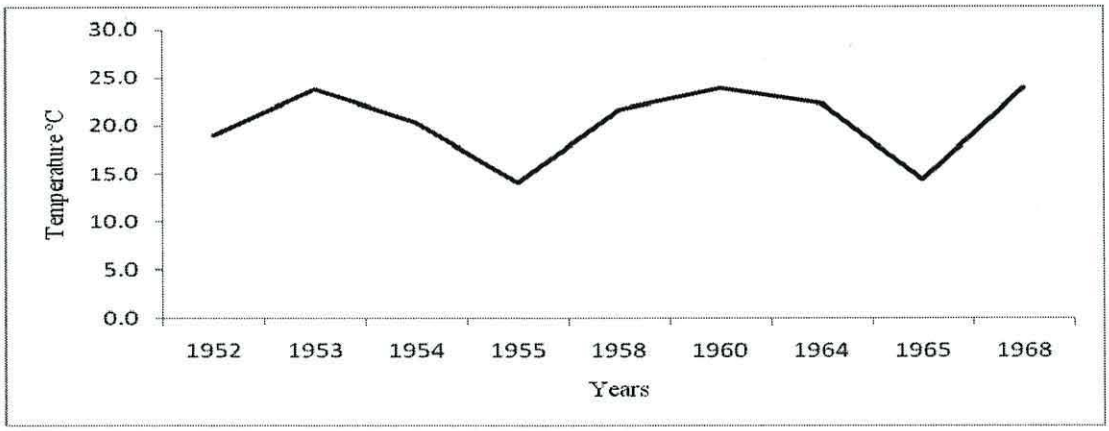


Figure 4.5 Mean annual temperature (°C) recorded at Otavi station in Otjozondjupa region, Namibia.

4.4.1.2 Ohangwena region

Mean annual rainfall between 1968 and 2003 (Figure 4.6) was 370 mm and mean annual temperature between 1944 and 1985 (Figure 4.7) was 23.3°C for Ohangwena region. An incredible decrease in mean annual rainfall was observed with a slight decrease in mean annual temperature during those specific time frames.

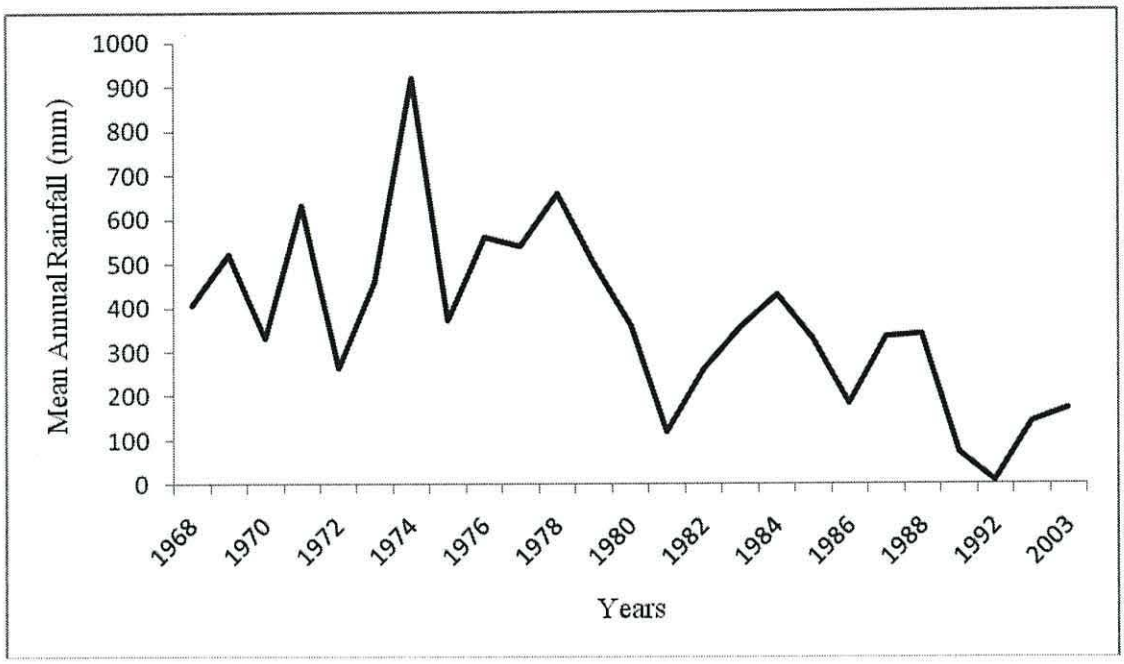


Figure 4.6 Mean annual Rainfall (mm) for Ondangwa in Oshana region, Namibia.

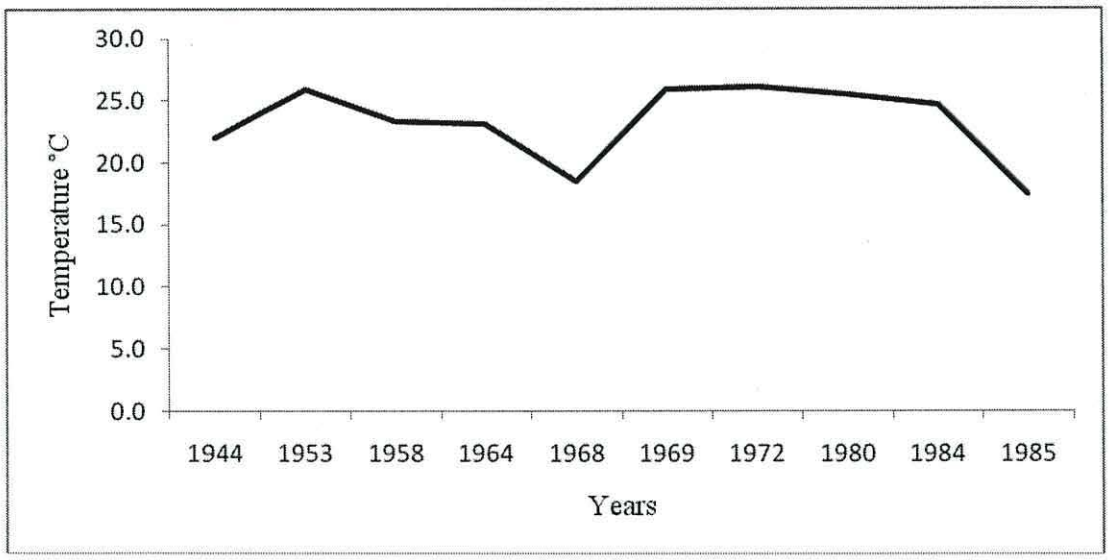


Figure 4.7 Mean annual temperature (°C) for Ondangwa in Oshana region, Namibia.

4.4.1.3 Omusati region

Records of average annual rainfall between 1991 and 2007 and temperature between 1972 and 1977 gave a mean annual rainfall of 454.9 mm and temperature of 23.1°C. A steady increase in mean annual rainfall was obtained in Omusati region between 1972 and 1977. (Figure 4.8 and 4.9, respectively).

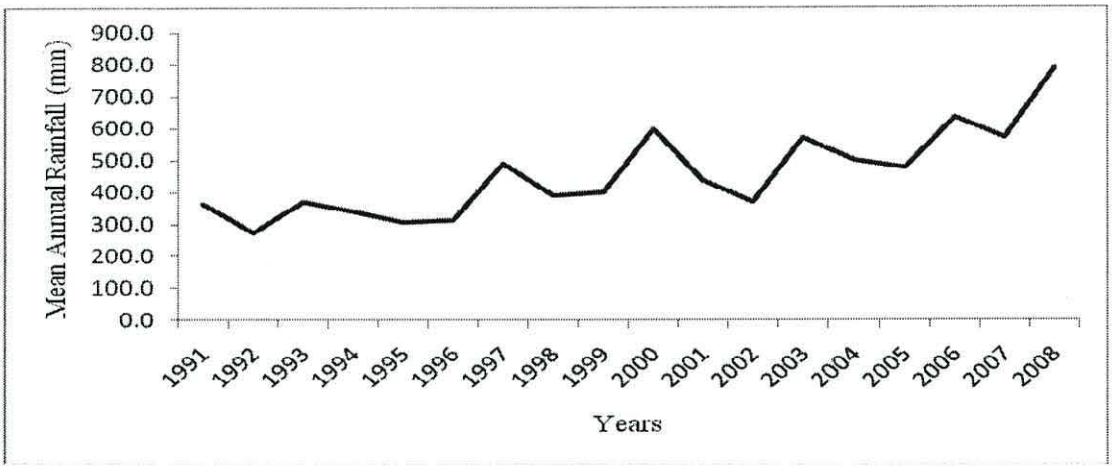


Figure 4.8 Mean annual Rainfall (mm) for Mahenene in Omusati region, Namibia.

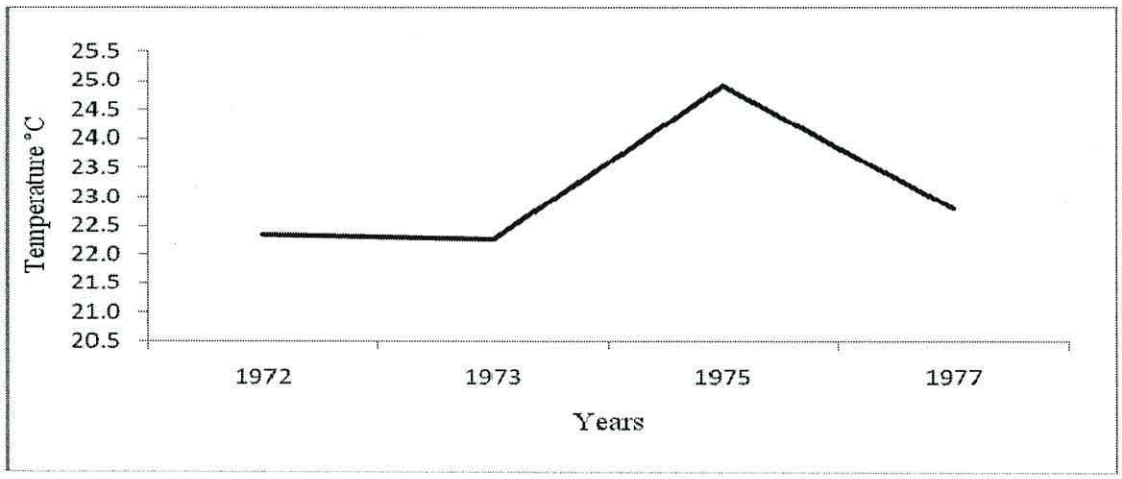


Figure 4.9 Mean annual temperature (°C) for Mahenene in Omusati region, Namibia.

4.4.2 Soil conditions

Analysis of soil texture indicated that sandy soil was the dominant soil type in all the study regions. Ohangwena region had, however, significantly higher ($P<0.001$) sand content (86.4±0.6%); and the lowest sand content was in Otjozondjupa region (48.5±

2.04%). In terms of clay and silt content, the highest (P<0.001) was in Otjozondjupa region (28.1±2.6% and 23.6±1.5, respectively); and the lowest in Ohangwena region (5.9±0.8%) and 7.8±0.7%, respectively) (Figure 4.10).

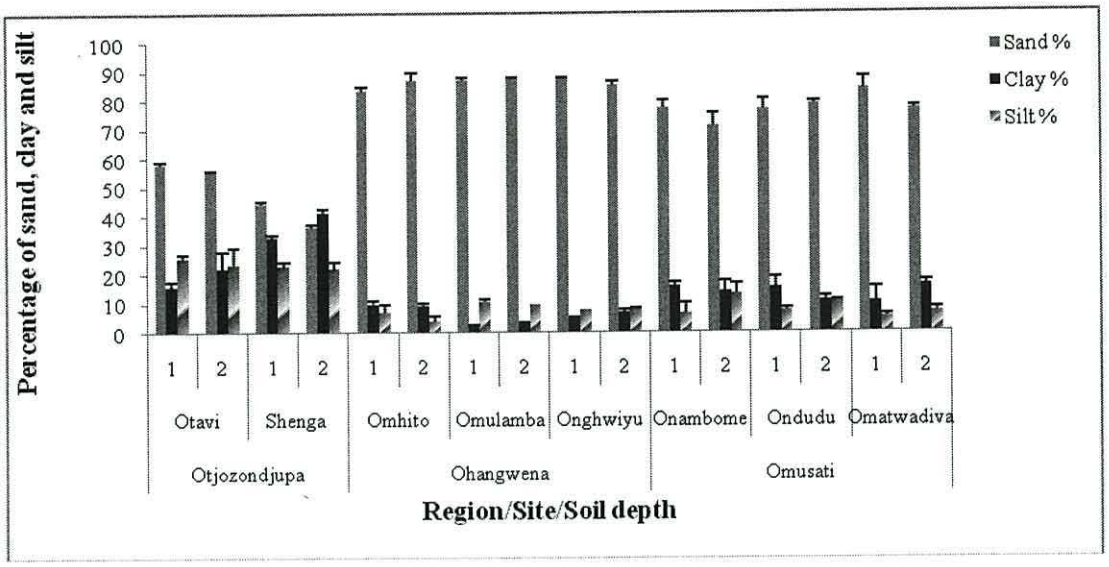


Figure 4.10 Mean percentage of soil conditions in Otjozondjupa, Ohangwena and Omusati regions, Namibia.

Analysis of soil nitrogen indicated that Otjozondjupa region had significantly higher (P<0.001) nitrogen content (0.1±0.0%); and the lowest content was in Ohangwena region (0.03± 0.0%). However there was no significant difference in nitrogen content between Ohangwena and Omusati regions (Figure 4.11).

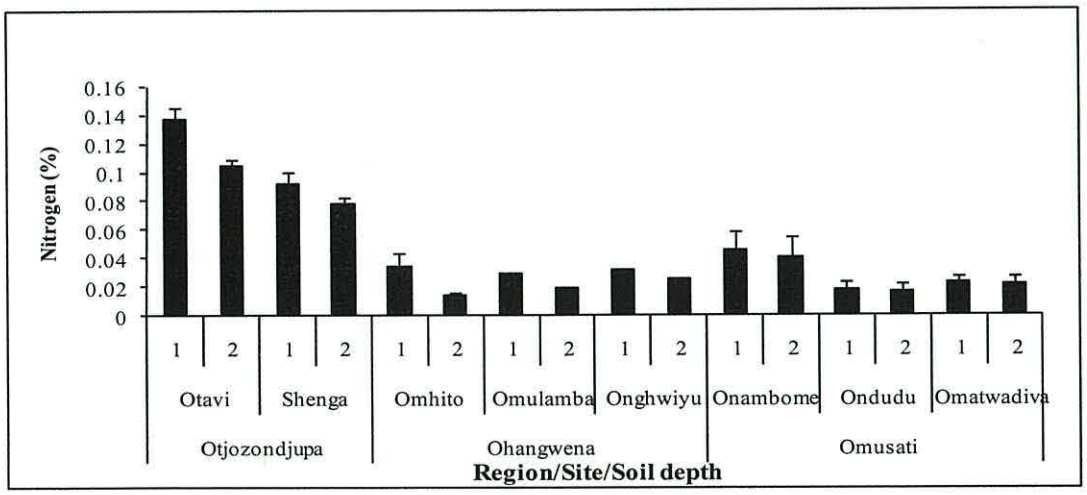


Figure 4.11 Mean percentage of soil nitrogen in Otjozondjupa, Ohangwena and Omusati regions, Namibia.

Significantly higher ($P<0.001$) soil organic matter content was found in Otjozondjupa region (2.1±0.2%) and the lowest content was in Omusati region 0.7± 0.1% (Figure 4.12).

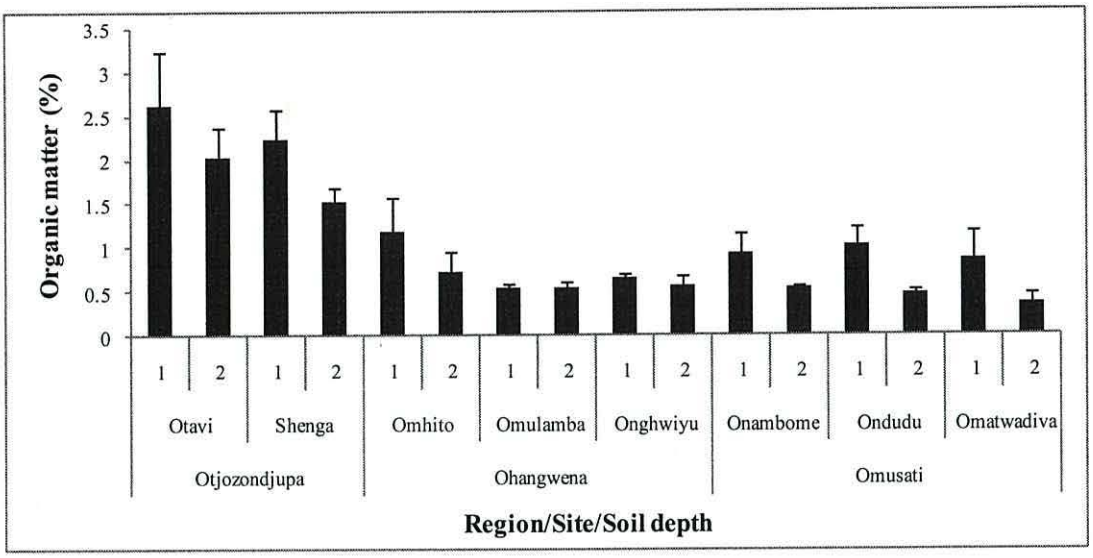


Figure 4.12 Mean percentage of soil organic matter in Otjozondjupa, Ohangwena and Omusati regions, Namibia.

There were no significant differences in phosphorus content (mg kg^{-1}) between the soils of the three regions (Figure 4.13).

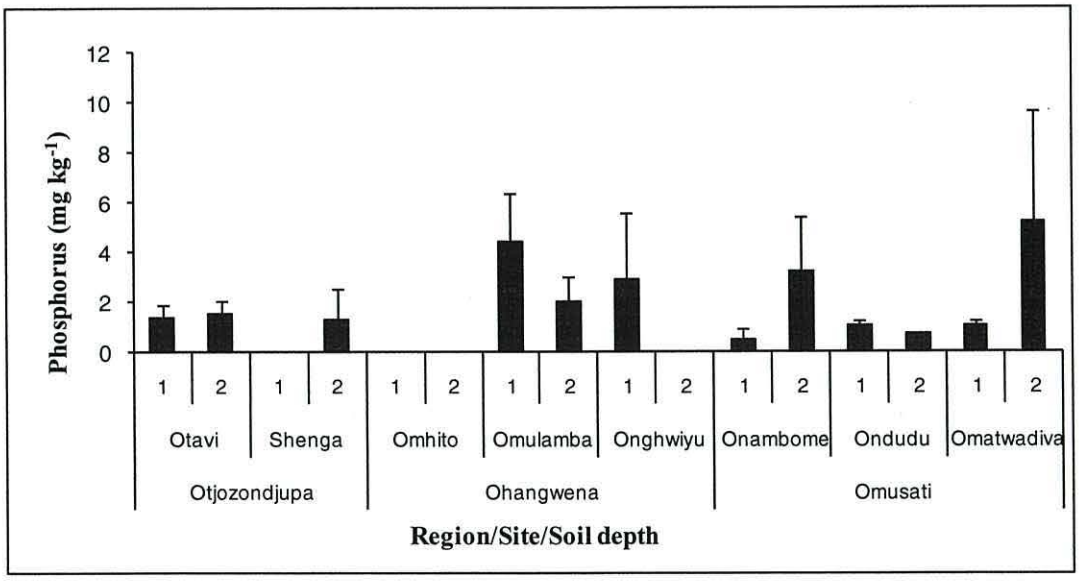


Figure 4.13 Mean amount of phosphorus in the soil in Otjozondjupa, Ohangwena and Omusati regions, Namibia.

Analysis of potassium, calcium, magnesium and sodium indicated that Omusati region had significantly higher ($P<0.001$) potassium (140.8±13.6 mg kg^{-1}), calcium (1697±422.7 mg kg^{-1}), magnesium (197.9±30.8 mg kg^{-1})and sodium (1956±877.2 mg kg^{-1}); and the lowest contents were found in Ohangwena region: potassium (57.3± 7.2 mg kg^{-1}), calcium (211± 32.3 mg kg^{-1}), magnesium (72.1± 10.8 mg kg^{-1}), sodium (6± 1.4 mg kg^{-1}) as illustrated in Figures 4.14, 4.15, 4.16 and 4.17, respectively.

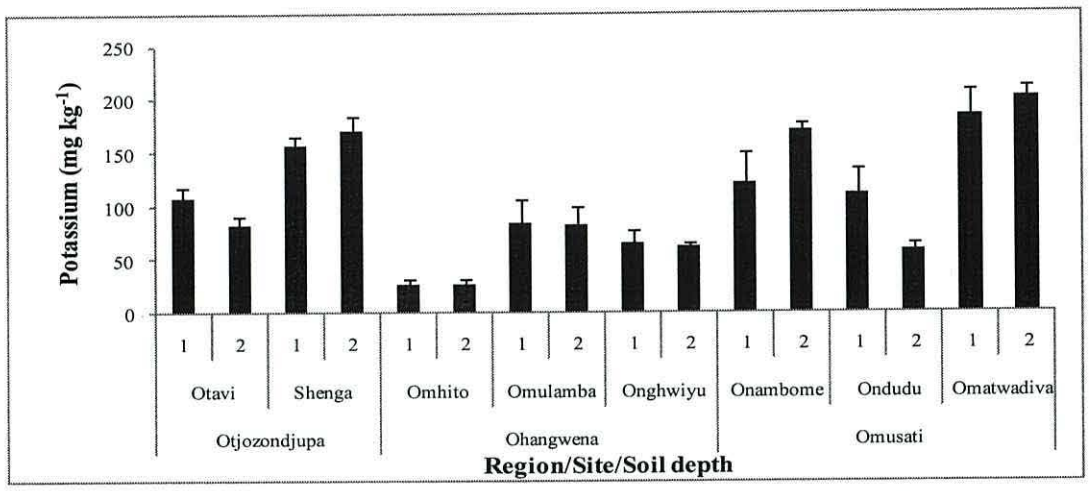


Figure 4.14 Mean amount of potassium in the soil in Otjozondjupa, Ohangwena and Omusati regions, Namibia.

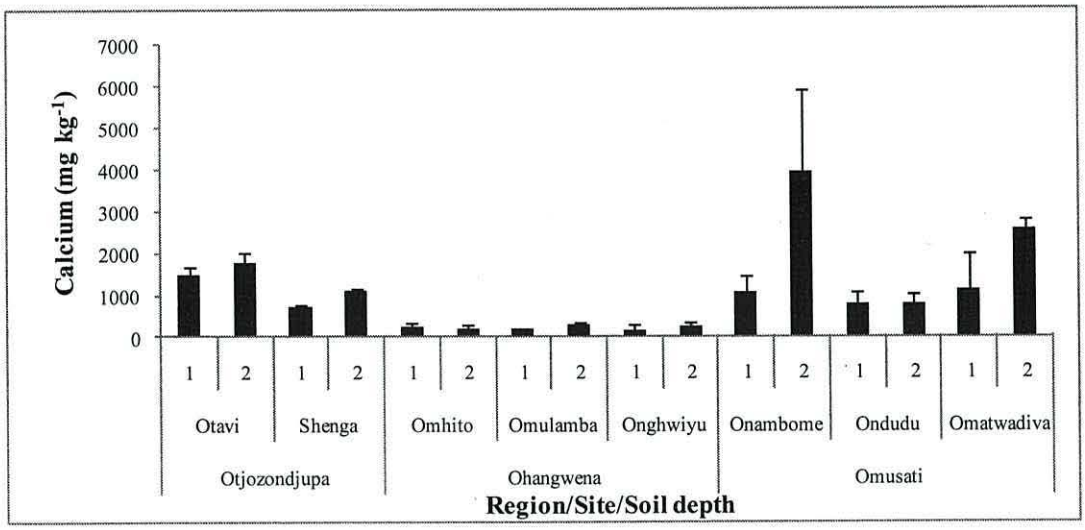


Figure 4.15 Mean amount of calcium in the soil in Otjozondjupa, Ohangwena and Omusati regions, Namibia.

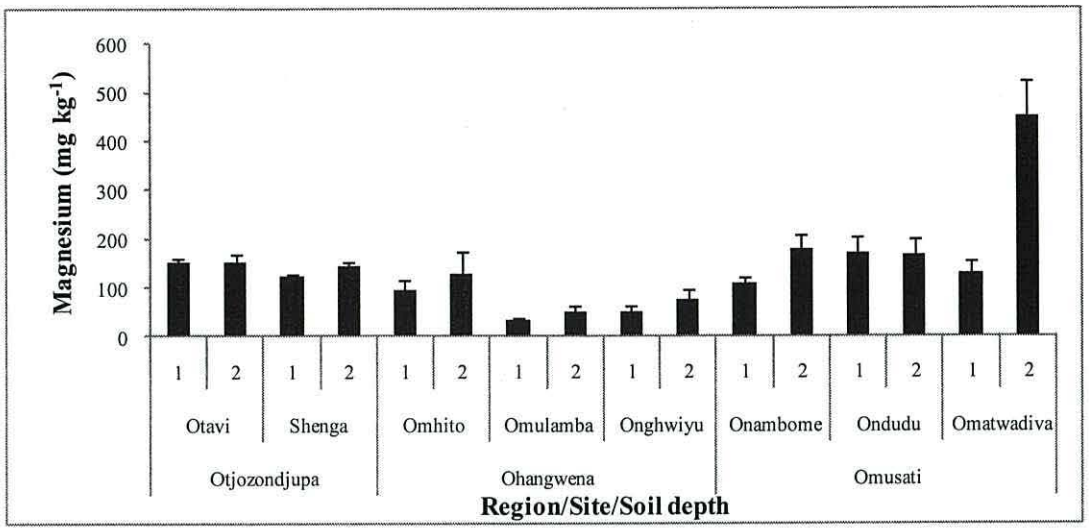


Figure 4.16 Mean amount of magnesium in the soil in Otjozondjupa, Ohangwena and Omusati regions, Namibia.

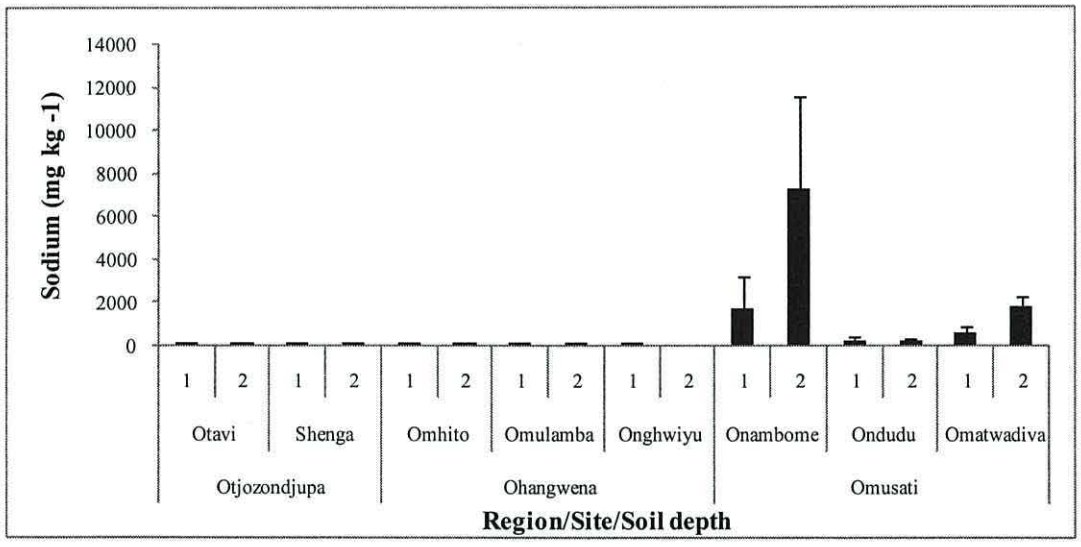


Figure 4.17 Mean amount of sodium in the soil in Otjozondjupa, Ohangwena and Omusati regions, Namibia.

Significant differences ($P<0.001$) in soil pH was found between regions, the highest being in Otjozondjupa (7.4±0.1) and the lowest in Ohangwena (6.4±0.1) (Figure 4.18). Analysis of soil electrical conductivity indicated a highly significant difference between sites ($P<0.001$), the highest being in Omusati region (607±161.6μS cm^{-1}); and the least in Ohangwena region (67± 7.7μS cm^{-1}) (Figure 4.19).

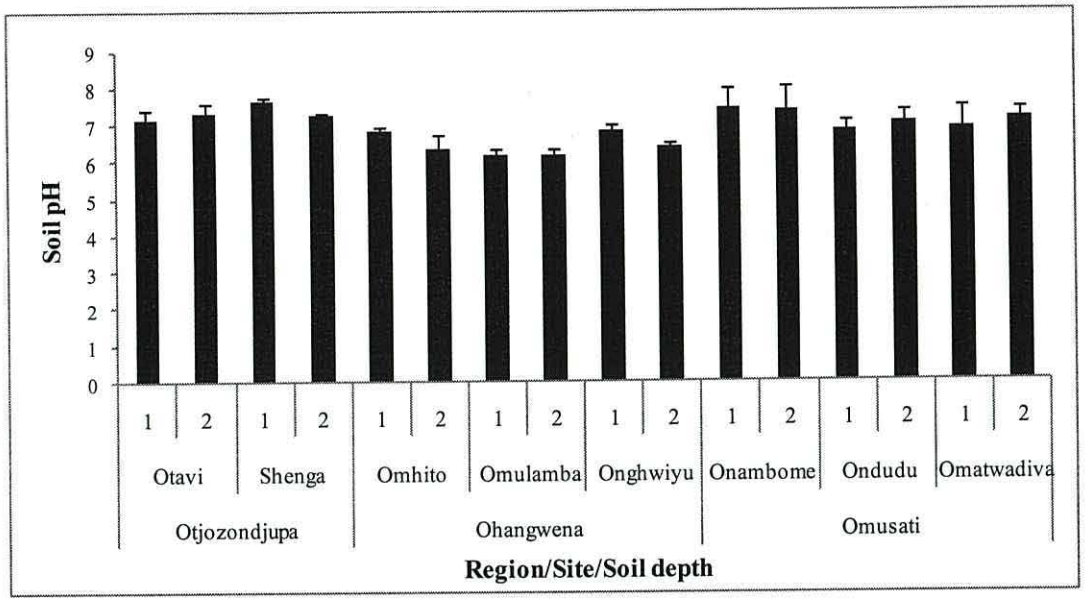


Figure 4.18 Soil pH in Otjozondjupa, Ohangwena and Omusati regions, Namibia.

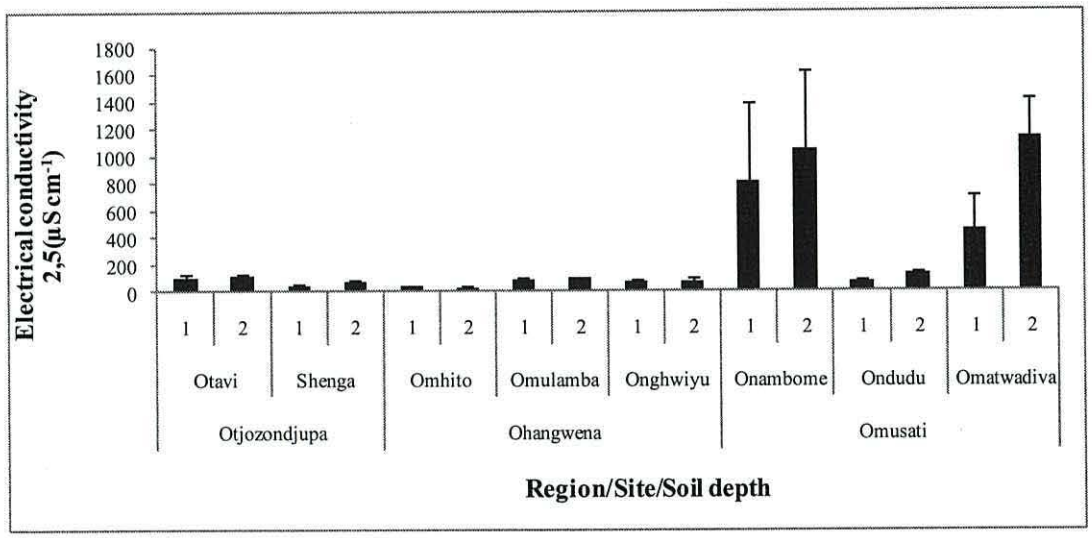


Figure 4.19 Soil electrical conductivity in Otjozondjupa, Ohangwena and Omusati regions, Namibia.

4.4.3 Population status, regeneration and associated plant species

4.4.3.1 Leaf categories and total number of plants per hectare

Results of the analysis of total number of plants per hectare per leaf category between regions indicated that there were no significant differences in the number of

plants between regions in the following leaf categories; 6 – 10, 11 – 15, 16 – 20 and 26+ leaf categories. There was, however, a significant difference ($P<0.001$) between the regions in the number of plants in the 21 – 25 leaf category; the highest was recorded in Ohangwena region (767±274.9 ha^{-1}) and the lowest in Omusati region (78±66.2 ha^{-1}) (Table 4.1).

Mean total number of *A. zebrina* plants of all leaf categories per hectare showed that the highest number (14800±3252 ha^{-1}) was recorded in Omhito village of Ohangwena region and the lowest in Omatwadiva village of Omusati region (3567±1217 ha^{-1}) (Table 4.1).

Table 4.1 Average number of *A. zebrina* plants per hectare per leaf category in Otjozondjupa, Ohangwena and Omusati regions, Namibia.

Region	Site	6-10	11-15	16-20	21-25	26+	Total
Otjozondjupa	Otavi	1200±167.3	1720±203.5	1500±288.1	340±92.7	380±182.8	5140±592.1
	Shenga	4960±977.0	1620±591.1	140±116.6	0±0.0	0±0.0	6720±1652.1
Ohangwena	Omhito	7867±1356.9	4333±1211.5	1867±664.2	700±300.0	33±33.3	14800±3251.7
	Omulamba	1267±352.8	1133±536.5	1533±887.6	1100±850.5	1433±796.5	6467±3146.6
	Onghwiyu	3033±1713.0	1500±360.6	900±305.5	500±0.0	133±88.2	6067±1732.4
Omusati	Onambome	6667±2172.8	3433±1649.6	1033±233.3	200±200.0	33±33.3	11367±3517.7
	Ondudu	3400±321.5	1233±448.5	167±88.2	0±0.0	100±100.0	4900±568.6
	Omatwadiva	2167±1066.7	1033±202.8	300±57.7	33±33.3	33±33.3	3567±1217.0

4.4.3.2 Plant height

Significant differences were found in *A. zebrina* plant height in different regions ($P<0.001$), the tallest being at Omulamba in Ohangwena region (40.4±2.1 cm); and the shortest at Shenga in Otjozondjupa region (22.8± 1.1 cm) (Figure 4.20).

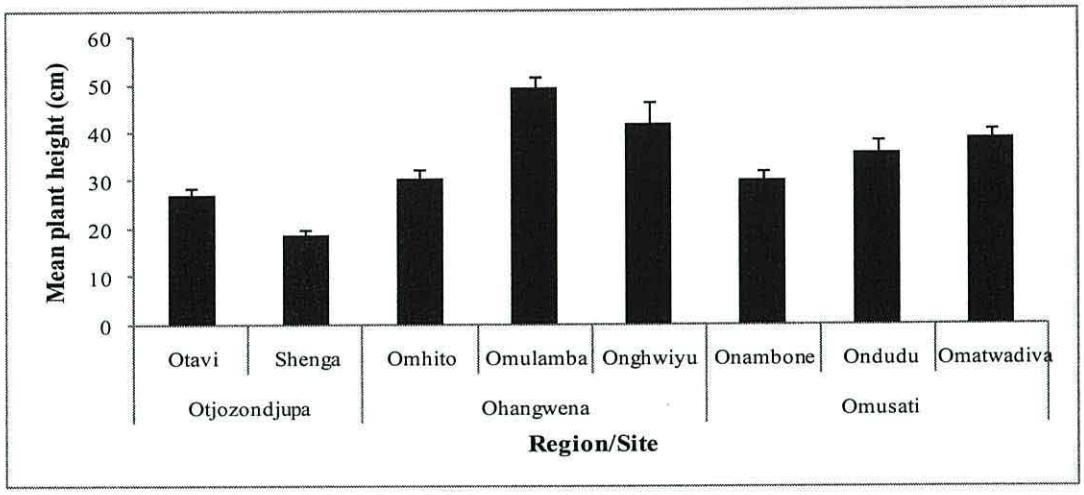


Figure 4.20 Mean plant height in Otjozondjupa, Ohangwena and Omusati regions, Namibia.

4.4.3.3 Plant diameter

Significant differences were found between mean plant diameter in different regions ($P<0.001$), the widest being at Omulamba in Ohangwena region (53.3±3.1 cm); and the narrowest at Shenga in Otjozondjupa region (34.3±1.4 cm) (Figure 4.21).

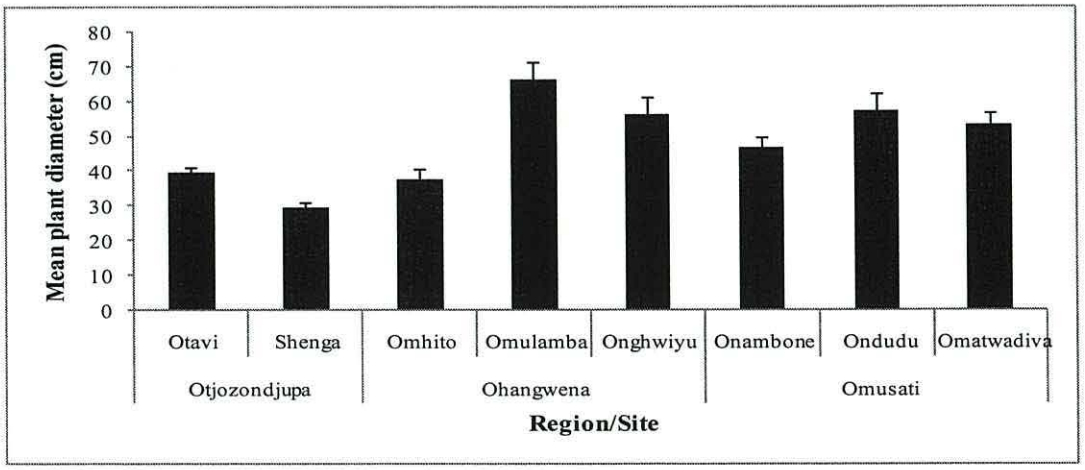


Figure 4.21 Mean plant diameter in Otjozondjupa, Ohangwena and Omusati regions, Namibia.

Analysis on the relationship between average plant height and average plant diameter indicated a strong positive linear relationship between the two variables (n = 138, P<0.001) (Figure 4.22).

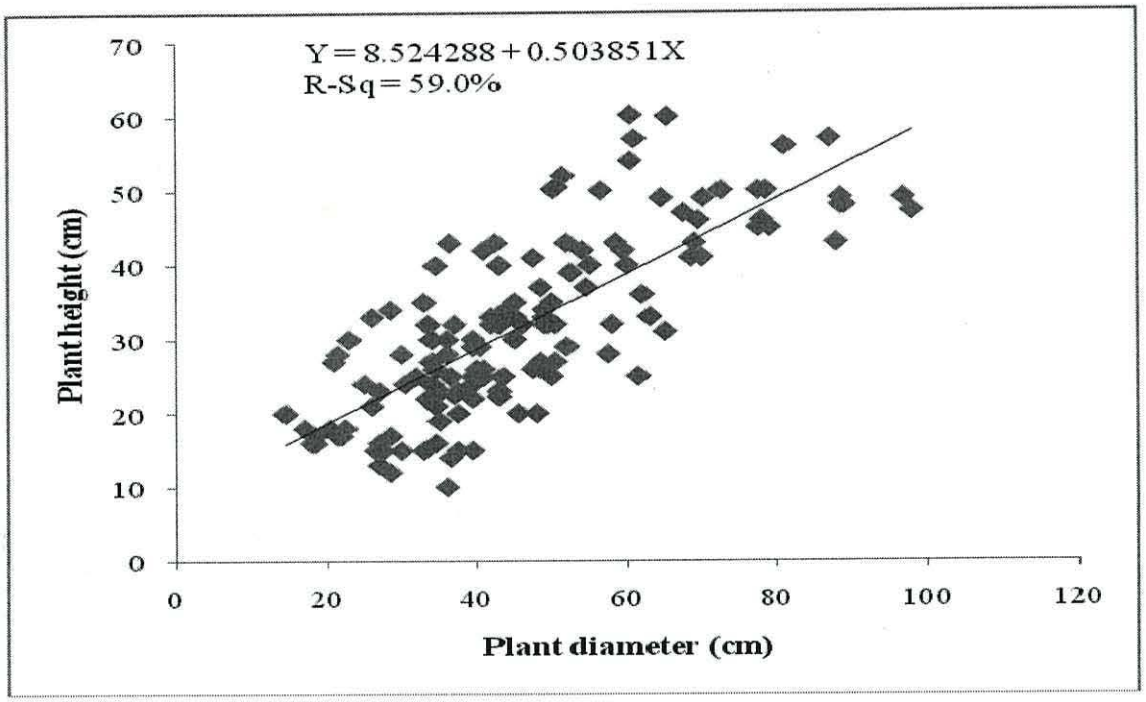


Figure 4.22 Regression analysis for average plant height verses plant diameter in Otjozondjupa, Ohangwena and Omusati regions, Namibia (r = 76.9, n = 138, P<0.001).

4.4.4 Regeneration

The highest number of seedlings regenerated from seeds per hectare was recorded in Ohangwena region and the lowest was in Omusati region, with no regeneration by seeds recorded in Otjozondjupa region. The highest regeneration by roots was recorded in Otjozondjupa region and the lowest in Ohangwena region (Table 4.2).

Table 4.2 Total number of *A. zebrina* seedlings per hectare regenerated from seeds and from roots in Otjozondjupa, Ohangwena and Omusati regions, Namibia.

Region	Regeneration by seeds	Regeneration by roots
Otjozondjupa	0	310
Ohangwena	311	189
Omusati	222	300

Mean total number of *A. zebrina* regeneration (1-5 leaves) per hectare indicated a significant difference between regions ($P<0.001$); the highest was in Omusati region and the least in Otjozondjupa region (Figure 4.23).

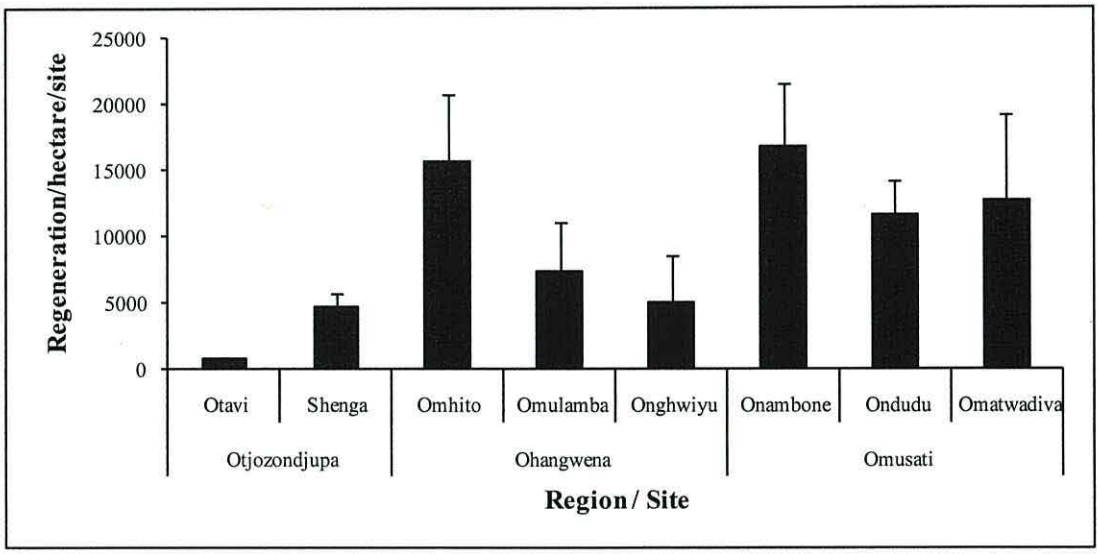


Figure 4.23 Total number of *A. zebrina* (1-5 leaves) regeneration between study sites in Otjozondjupa, Ohangwena and Omusati regions, Namibia.

4.4.5 Associated plants

Findings on *A. zebrina* associated plant species (trees and shrubs) were identified and recorded in each study site. Only two species were common to the three study sites: *Euclea divinorum* and *Grewia* sp. (Table 4.3). The highest number of associated plant species was observed in Ohangwena region and the lowest in Omusati region.

Similarities in associated species between sites were assessed by determining Sørensen's similarity index. The result shows that the Sørensen's index of similarity between study sites ranged from 0.00 to 0.33, which indicates dissimilarity between sites in plant species associated with *A. zebrina* (Table 4.4).

Table 4.3 Associated plant species recorded per study site in Otjozondjupa, Ohangwena and Omusati regions, Namibia.

Region	Otjozondjupa			Ohangwena				Omusati				Common to all sites
	Sites			Sites				Sites				
Species	Otavi	Shenga farm	common	Omhito ya Nanime	Omulamba	Onghwiyu	common	Onambome	Ondudu	Omatwadiva	common	
Acacia erioloba	0	0	0	1	1	0	0	0	0	0	0	0
Acacia mellifera	1	1	1	0	0	0	0	1	0	0	0	0
Acacia nebrownii	0	0	0	0	0	0	0	1	0	0	0	0
Acacia senegal	1	0	0	0	0	0	0	0	0	0	0	0
Acacia sieberiana	0	0	0	0	0	0	0	0	1	0	0	0
Aloe esculenta	0	0	0	0	0	0	0	0	1	0	0	0
Baikiaea plurijiga	0	0	0	1	0	0	0	0	0	0	0	0
Baphia massaiensis	0	0	0	1	0	1	0	0	0	0	0	0
Berchemia discolor	0	0	0	0	1	0	0	0	0	0	0	0
Boscia albitrunca	0	0	0	1	0	0	0	0	0	0	0	0
Colosphospermum mopane	0	0	0	0	0	0	0	1	1	1	1	0
Combretum collinum	0	0	0	1	0	1	0	0	0	0	0	0
Combretum hereroense	1	0	0	0	0	0	0	0	0	0	0	0
Combretum imberbe	1	0	0	0	0	0	0	0	0	0	0	0
Combretum zeyheri	0	0	0	1	0	1	0	0	0	0	0	0
Commiphora africana	1	1	1	0	0	1	0	0	0	0	0	0
Dichrostachys cinerea Africana	1	1	1	1	0	1	0	0	0	0	0	0
Ehakanhoni (nhoni)	0	0	0	0	0	0	0	1	0	0	0	0

Erythrophleum africanum	0	0	0	1	0	0	0	0	0	0	0	0
Euclea divinorum	1	0	0	1	0	0	0	1	0	0	0	1
Grewia flavescens	0	1	0	1	0	1	0	0	0	0	0	0
Grewia sp.	1	1	1	1	0	1	0	0	1	0	0	1
Hyphaene petersiana	0	0	0	0	1	0	0	1	0	0	0	0
Lonchocarpus nelsii	0	1	0	0	0	1	0	0	0	0	0	0
Maytenus senegalensis	1	0	0	0	0	0	0	0	0	0	0	0
Muendulea sericea	0	0	0	0	0	1	0	0	0	0	0	0
Ochna pulchra	0	0	0	1	0	0	0	0	0	0	0	0
Okambango	0	0	0	0	0	1	0	0	0	0	0	0
Oluputa	0	0	0	0	0	1	0	0	0	0	0	0
Ombango	0	0	0	1	0	1	0	0	0	0	0	0
Oshivatu	0	0	0	0	0	1	0	0	0	0	0	0
Peltophorum africanum	0	1	0	0	0	0	0	0	0	0	0	0
Rhus tenuinervis	1	0	0	1	0	1	0	0	0	0	0	0
Terminalia prunoides	1	0	0	0	0	0	0	0	1	0	0	0
Terminalia sericea	0	0	0	1	0	0	0	0	0	0	0	0
Vangueria infausta	0	0	0	0	1	1	0	0	0	0	0	0
Ximenia americana	0	0	0	1	0	1	0	0	0	0	0	0
Zizyphus mucronata	0	0	0	1	0	1	0	0	0	0	0	0
Total	11	7	4	17	4	17	0	6	5	1	1	2

Table 4.4 Sørensen's similarity index for all pairs of study sites from Otjozondjupa, Ohangwena and Omusati region, Namibia

	Otavi muni plots	Shenga farm	Omhito ya Nanime	Omulamba	Onghwiyu	Onambome	Ondudu	Omatwadiva
Otavi muni plots		0.17	0.14	**0.00**	0.03	0.01	0.05	**0.00**
Shenga farm	0.17		0.15	**0.00**	0.22	0.08	0.07	**0.00**
Omhito ya Nanime	0.14	0.15		0.07	**0.33**	0.06	0.06	**0.00**
Omulamba	**0.00**	**0.00**	0.07		0.06	0.14	**0.00**	**0.00**
Onghwiyu	0.03	0.22	0.33	0.06		**0.00**	0.05	**0.00**
Onambome	0.01	0.08	0.06	0.14	**0.00**		0.10	0.09
Ondudu	0.05	0.07	0.06	**0.00**	0.05	0.10		0.08
Omatwadiva	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.09	0.08	

4.4.6 Relationships between soil characteristics of sites and morphological characteristics of *A. zebrina*

As shown in Table 4.5, there was no relationship between soil mineral contents and plant morphological parameters. There were, however, significant and positive relationships between the sand content of soil and height and diameter of *A. zebrina* (Table 4.7). There were also significant but negative corrections between height and diameter of *A. zebrina* and silt, clay, N and OM contents of soil (Tables 4.6 & 4.7). There was a significant negative relationship between pH and height of *A. zebrina* but not with diameter (Table 4.6).

Table 4.5 Pearson correlations between soil mineral contents and plant morphological characteristics

	P ppm	K ppm	Ca ppm	Mg ppm	Na ppm
Plant height	0.21	-0.23	-0.18	-0.18	0.09
	0.27	0.24	0.37	0.35	0.67
Plant diameter	0.31	-0.11	-0.11	-0.07	0.19
	0.11	0.56	0.59	0.73	0.33

Table 4.6 Pearson correlations between soil chemical properties and plant morphological characteristics

	N %	pH	EC	OM %
Plant height	-0.6	-0.4	0.2	-0.6
	0.0	0.0	0.4	0.0
Plant diameter	-0.5	-0.2	0.2	-0.5
	0.0	0.2	0.2	0.0

Table 4.7 Pearson correlations between soil texture and plant morphological characteristics

	Sand %	Clay %	Silt %
Plant height	0.74	-0.7	-0.6
	0.0	0.0	0.0
Plant diameter	0.6	-0.7	-0.5
	0.0	0.0	0.0

4.5 Discussion

4.5.1 Ecological conditions and population status of *A. zebrina*

The climate data collected from the three study sites showed that the mean annual rainfall was ranged between 370 and 472 mm, despite the species rainfall range of 500-625 mm per annum reported (Jeppe, 1969; SEPASAL, 2007) while Backyardgardener (2008) reported semi-arid climate (300-500 mm) as the species' major ecological requirement. Mean annual temperature ranged between 20.4 and 23.1°C, despite species preferred temperature of 10°C as reported (Faucon, 2005; SEPASAL, 2007), both of which are characteristic of dryland ecosystems reported to be the ecological requirements for *A. zebrina*. This is an indication that the species can grow in wide range of ecological conditions, ranging from mean annual rainfall of 370-625 mm and mean annual temperature of 20-23°C and it can tolerate low temperature as 10°C.

The results of soil analysis indicated that sandy soil is the dominant soil type in all the study regions, with Ohangwena region showing significantly the highest sand content (86%); and the least was in Otjozondjupa region (49%). There was a strong and positive correlation between plant parameters and sand content of soil. The highest number of plants per hectare and large sized plants in terms of height and diameter were found in Ohangwena region where soil sand content was the highest, implying that sandy soil is the most suitable soil condition for *A. zebrina*. This is in line with *A. zebrina*'s favourable soil growing condition reported by Rothmann (2004), SEPASAL (2007) and Backyardgardener (2008). The lowest number of plants per hectare and the smallest sized plants in terms of height and diameter were found in Otjozondjupa region, perhaps because of low sand and high organic matter and N contents in the soil of this region. In terms of clay content (28%) and silt content (24%), the highest was recorded in Otjozondjupa region as well as the highest soil nitrogen content (0.1%) and organic matter content (2%); and the least clay (6%) and silt content (8%) recorded in Ohangwena region.

Concerning mineral contents, there was no significant difference in phosphorus content (mg kg^{-1}) between the sites and the amount was extremely low. As reported by Busman *et al.* (2002), coarse-textured sandy soils generally hold much less phosphate due to the more still character of sand particles as compared to clay particles. Since sandy soil was the dominant soil in the study area, this may explain why there was less phosphorus content in these sites. For potassium, calcium, magnesium and sodium (mg kg^{-1}), however, Omusati region had the highest contents of all four elements and the lowest were recorded in Ohangwena region. This shows that the higher the sand content of soil, the lower is the mineral contents due to very low cation holding capacity of sandy soils (Busman *et al.* 2002). Analysis of the soil pH indicated a significant difference between regions; the highest was in Otjozondjupa (7.3) and the lowest was in Ohangwena (6.4). This may also explain why there were fewer and smaller sized plants in Otjozondjupa region where the pH is out of the range of pH required by *A. zebrina* (5.5-6.5 pH) according to SEPASAL (2007) and Backyardgardener (2008).

Analysis of soil electrical conductivity indicated a highly significant difference between the sites, the highest being in Omusati region (607.4 µS cm^{-1}); and the lowest in Ohangwena region (67.1 µS cm^{-1}). As documented by Grattan (2002), electrical conductivity depends on the level of cations in the soil and every crop has a level of soluble salts that it can tolerate in the soil solution (the salinity threshold), above which the plant will experience yield reduction. That means *A. zebrina* may have a very low salinity threshold of around 67.1 µS cm^{-1} because this was value recorded for Ohangwena region where it was found growing abundantly and achieving large size.

4.5.2 Regeneration and associated plant species

Significant differences were found between regions in mean total number of *A. zebrina* regeneration per hectare; the highest was in Omusati region and the lowest in Otjozondjupa region. In terms of the method of regeneration, however, the results showed that the highest *A. zebrina* regeneration from roots was recorded in Otjozondjupa region, with no plants regenerated from seeds. On the other hand, in Ohangwena region most regeneration was from seeds and the lowest number of plants regenerated from roots was recorded. The low number of regeneration from seeds in

Otjozondjupa region may be due to overharvesting of flowers (Chapter 3) suggesting this practice is unsustainable and may threaten the species with extinction. As documented by Grace *et al.* (2008) unsustainable harvesting for natural products is one of the major threats to *Aloes* worldwide. Similar comment was also made by Belem *et al.* (2008) in Burkina Faso's Central Plateau, that unsustainable harvesting of *Bombax costatum* flowers is depriving the species of large quantities of seeds for regeneration. Similar observations were also reported by Ouédraogo *et al.* (2006) in Burkina Faso where *Bombax costatum* and *Boswellia dalzielii* populations showed a strong decline characterized by aging trees and difficulties in the regeneration, establishment and development of saplings. Although regeneration by root suckers was observed, slow growth of saplings was the main obstacle to their regeneration in a disturbed semi-arid area.

The results on *A. zebrina* associated plant species (trees and shrubs) showed dissimilarities in associated species between sites. There were only two associated plant species which were common to all the three study regions. These may be due to variation in the soil characteristics and land use intensity between the sites. The highest number of associated plants was observed in Ohangwena region where high density of mature plants and regeneration of *A. zebrina* was also recorded. This may be due to less intensive land use and exploitation of plant species in this region (Chapter 3).

CHAPTER 5

NUTRITIONAL COMPOSITION OF *ALOE ZEBRINA* BAKER

This chapter deals with the determination of nutritional composition of *Aloe zebrina* cooked and dried flower products. This chapter is divided into four sections. In the first section, background information on the state of knowledge and a brief review of this research was presented. In the second place, description of procedures followed in sampling flowers, processing, appropriate laboratory analysis and objectives of present research were given. This is followed by analytical methods used in determining crude protein, dietary fibre, lipids, ash, vitamins and minerals. The third and fourth sections presented the results and discussion, respectively.

5.1 Introduction

The importance of *A. zebrina* for food has been documented by several previous workers such as Jansen (2005) and Lefers (2003) who reported that the reddish flowers of *A. zebrina* are edible, and flowers are boiled and eaten either straight as relish or processed into cakes, which are sun-dried and then eaten. Leger (1997) added that these red flowers are plucked when the plants are in full flower and cooked together with meat or fish, which results in a red sauce with a tomato-like taste, sometimes referred to as 'tomato sauce'. Li (2009) reported that many *aloes* have cosmetic, medicinal, ornamentals and food values, and the edibles aloes have all kinds of physical nutrition necessary to humans. The edible aloes can be consumed as a vegetable and processed into food or beverages. These foods are not only nutritious but also have their own unique flavour. According to him, there are only six *Aloe* species that are edibles (not listed).

According to Özbucak *et al.* (2007) wild plants have been used as a source of food ever since humans existed. Many edible wild plants have been used as salad, vegetables, fruits, and spices in fresh, raw or dried form (Özbucak *et al.* 2007; Redzic, 2006). Gari (2003) has documented that wild food plants are relevant in household food security and

nutrition in some rural areas particularly in dry lands, and therefore represent important food sources during seasonal food shortage periods by providing nutritional supplies especially micronutrients. The nutritional composition of many wild food plants including *A. zebrina* is, however, not known and therefore their significance in the nutrition of local communities is un-quantified.

The importance of *A. zebrina* for food, medicine and feed for animals, combined with the lack of information on the nutritional composition of the species necessitated the current study. This study represents the first attempt to establish the nutritional value of the species. The present research was carried out to determine the nutritional composition of edible cooked and dried *A. zebrina* flowers through laboratory analysis of crude protein, dietary fibre, ash, lipids, vitamins and minerals including calcium, potassium, magnesium, phosphorus, sodium, iron, manganese and zinc. These nutritional components were chosen because of their importance in human nutrition as described below.

Proteins are a large group of organic compounds found in all living organisms and consist of one or several long chains of amino acids (Brunetti, 2004). Different proteins perform different functions in the body such as helping in the formation of antibodies that enable the body to fight infections, serving as major energy supplies and generally repairing, regulating and protecting our bodies. Dietary fibre is the term used to describe several materials that make up the parts of carbohydrates that the body cannot digest. It is regarded as an essential component of a healthy diet; it is known for keeping regular bowel movement and prevents intestinal problems. It has also been reported that foods which are rich in fibre help in lowering the risk of chronic conditions such as heart disease, cancer as well as type 2 diabetics. Fibre is generally filling and relatively low in calories. Fibre is found in plant parts that are eaten for food, such as grains, legumes, fruits and vegetables (AHA & Ward, 2008; Roizen, 1997).

The "**ash content**" is a measure of the total amount of minerals present within a food. In other words, ash is the inorganic residue remaining after water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food.

Lipids, one of the major components of foods, are important in human diet for various reasons, such as being a major source of energy and providing essential lipid nutrients. Too much of some lipids components, for example cholesterol and saturated fats can, however, be harmful to human health, (McClements, 2008). McClements further claimed that in many foods, the lipid component plays a major role in determining the overall physical characteristics of foods, for example flavour, texture, taste and appearance. That is why it is difficult to develop low-fat alternatives of most foods; simply because once the fat is removed some of the most important physical characteristics are lost. Lipids in foods are those components which are soluble in organic solvents (such as ether, hexane or chloroform), but are insoluble in water. The terms fat, oil and lipid are often used interchangeably by food scientists, although sometimes the term fat is used to describe those lipids that are solid at the specified temperature and the term oil is used to describe those lipids that are liquid at the specified temperature (McClements, 2008).

According to Down (2008), **vitamins** are groups of essential nutrients which are required for different roles in the human body. They are divided into two groups, fat and water soluble vitamins. Any excess of fat soluble vitamins such as A. D, E and K is stored in the liver for later use, while the water soluble vitamins circulate in bloodstream, and pass through the body far more quickly than the fat soluble vitamins and are then excreted in the urine. The water soluble vitamin group embraces vitamin C and the eight major B-complex vitamins (B1, B2, B3, B5, B6, B7, B9 and B12).Water soluble vitamins are not able to be stored by the body unlike fat soluble vitamins (Sullivan, 1999). In the present experiment the following vitamins of the B complex were analysed.

Vitamin B1 (Thiamine): Thiamine is a required coenzyme / helper molecule in the metabolism of protein, carbohydrates and fats into energy, and proper transmission of nerve signals, for normal muscle function, growth, digestion, DNA replication as well as for normal appetite. It aids detoxification, heart function and the health of the nervous system. Sources of Vitamin B1 are sunflower seeds, wheat germ, whole grains, legumes, poultry, fish, pork, milk and green vegetables.

Vitamin B2 (Riboflavin): Riboflavin is necessary in the release of cellular energy production from carbohydrates, supports hormone production, neurotransmitter function, healthy eyes and skin, activation of many vitamins, breakdown of fat, for normal growth and tissue repair, production of red blood cells, corticosteroids and glycogen. It is obtainable from dairy products such as milk, yogurt and cheese, beef, broccoli, cocoa, eggs, pasta and green leafy vegetables.

Vitamin B3 (Nicotinic acid): Vitamin B3 is required by all cells and is vital in the release of energy from food, required for the synthesis of proteins, fat and genetic material, for proper metabolism and brain function. It has a positive influence on cholesterol levels. Vitamin B3 is found in tuna fish, halibut, poultry, whole grains & enriched breads, fortified cereals, seeds, milk, egg, legumes and peanut butter.

Vitamin B6 (Pyridoxine): B6 Vitamin is needed for proper protein synthesis, hormones, red blood cells, fatty acids, enzymes, conversion of tryptophan to niacin, it is needed for normal growth, supports brain function and skin health and is crucial for the immune system, it supports cardiovascular health by supporting homocysteine metabolism. Vitamin B6 is found in potatoes, sweet potatoes, milk, poultry, salmon fish, bananas, beans, walnuts, watermelon, vegetables, whole grain cereals and egg yolks.

Minerals are inorganic substances found in rocks and ore that find their way into our diets via plants, and animals that have fed on the plants (Ursell, 2001). According to Sullivan (1999) there are about 18 known minerals required for the maintenance of our bodies, and without minerals, vitamins would not be able to be assimilated. Sullivan (1999) further claimed that vitamins, minerals and other trace elements work together within the body to ensure that all processes can be carried out, such that with even a single element missing; the body can become unbalanced and unable to work at optimum level. In the present research, the following minerals were analysed.

Potassium (K): Potassium is important for muscle contraction and relaxation, nerve conduction, regulating heartbeat and healthy blood pressure. Sources of Potassium are fresh and dry fruits, peaches, avocado, raisins, dark green leafy vegetables, mushrooms, potatoes, peas, melons, banana, figs, legumes, meat, fish, wheat bran and dairy products.

Magnesium (Mg): According to Hands (2000) about two-thirds of all magnesium in the human body is found in bones. Magnesium is also found in the muscles and in other cell types and body fluids. As with all minerals, the body cannot make magnesium which means it must be obtained from food eaten. Mg also plays an important role in energy metabolism, muscles contraction and transmitting nerve impulses. It is needed for nearly 300 enzymes used in metabolic reactions in a human body, including synthesizing proteins and fatty acids as well as metabolizing glucose. It also helps in controlling the calcium balance in the human body. Rich sources of Magnesium are dairy products, breads and cereals, tofu, chocolate, nuts and seeds, soybeans, meat, fish, poultry, eggs, legumes and dark-green leafy vegetables.

Phosphorus (P): Phosphorus is important for a number of biochemical reactions in the body, especially energy production, metabolism of proteins, carbohydrates and fat, for healthy bones, teeth and is a component of all soft tissues and cell membranes. It also helps maintain the pH balance of the blood, muscles contraction, kidney function, proper nerve function and helps activate the B vitamins. Phosphorus is mainly obtained from animal proteins such as beef, chicken, pork, milk and milk products, fish eggs, also from plant products for instance nuts, legumes, cereals, grains, chocolate, lettuce and tomatoes.

Sodium (Na): Sodium is needed for the regulation of water balance within the body, maintenance of a normal body pH, plays a role in the generation of normal electrical nerve signals, muscle contraction as well as the regulation of blood pressure. Sources of Sodium are such as table salt, cheese and bread.

Calcium (Ca): Calcium is required for developing and maintaining healthy muscles, bones, teeth and gums. It also plays a major role in maintaining normal heart functions, clotting blood as well as muscle functions. Calcium is found in large amounts in dairy products, but also in turnip and mustard greens, kale, soybean, salmon fish with bones, clams, shrimp, amaranth and tofu.

Iron (Fe): Iron carries oxygenated red blood cells, support immune system, and is also important for a healthy pregnancy. Sources includes red meat, clams, oysters, green leafy vegetables, tofu, nuts, whole grain including enriched breads and cereals, dried fruits, legumes.

Zinc (Zn): Roizen (1997) documented that zinc is a component of several enzymes and also helps in protein synthesis, blood glucose regulation, wound healing and brain functioning. It also aids with maintaining healthy skin, immune, nervous, digestive and reproductive functions, as well as maintaining vitamin A levels in the blood. Zinc is reported to be important for proper growth of skin, hair, nails, detoxifying the body and is also responsible for carbohydrates metabolism. Main sources of Zinc are meats, seafood, eggs, legumes, green leafy vegetables, oysters, pork, poultry, milk, nut, whole grains including bran, wheat germ, sunflower seeds and broccoli.

Manganese (Mn): Manganese helps in the formation of connective tissues and bones, supports healthy brain function and reproduction, helps with energy production and is needed for normal glucose metabolism. Sources of Manganese are wheat germ, oatmeal, egg yolks, nuts, pineapple and green leafy vegetables (Roizen, 1997).

5.2 Materials and methods

5.2.1 Flower sample collection and processing

Nutritional composition of *A. zebrina* Baker was determined by analyzing cooked flowers and sun dried cakes. Fresh flowers were collected from Otavi surrounding in Otjozondjupa Region. Generally, in this region *A. zebrina* flowers are harvested by women with well trained fingers, collecting flowers into hessian bags (Figure 5.1), removing blossoms and buds of all the multi-branched panicles which have started to turn from green to red. Every twig or piece of green stem is removed and the product is properly searched for debris and beetles before cooking. Collection of flowers can be a challenge; since *A. zebrina* grow among bushes which may be very thorny (Figure 5.2). Harvested flowers are then cooked and processed into either cake or 'spinach'

(Volkmann, 1990). The same procedure was followed by the researcher to collect and process the samples used for the present study.

Figure 5.1 Harvesting of *A. zebrina* flowers using bulging hessian bags

Figure 5.2 Harvesting *A. zebrina* flowers among thorny *Acacia* species

5.2.2 Laboratory analysis

Laboratory analysis was conducted at Bangor University to determine the nutritional composition of the species. The analytical methods used are described below.

5.2.2.1 Crude Protein

In most plant materials, the crude protein of food stuff is estimated indirectly by first determining the percentage nitrogen content and then multiplying it by 100/16 = 6.25 to obtain an approximate percentage protein content. The factor 6.25 is derived from the basis that most proteins contain an average 16% of nitrogen (McClements, 2003; Brunetti, 2004; MoA, 2008). Commonly this is done is by converting the N in the sample to ammonia by digestion with acid in the presence of a catalyst. The amount of ammonia is then determined by titration against an acid using an indicator. This is referred to as the Kjeldahl method (McClements, 2003; MoA, 2008).

200 mg of dried *A. zebrina* flower sample which was placed into each of four tubes. One catalyst tablet and 5 ml concentrated sulphuric acid were added to each tube. Tubes were placed in a fume cupboard at 420°C for 20-30 minutes for digestion and left to cool. During the digestion, the nitrogen in the sample was converted into ammonia in the form of ammonium ion $NH4^{+}$ which binds to $SO4^{2}$ ions of the acid. After the digestion, the sample solution was placed in the Kjeltec analyser unit which determined its N content. The N content was multiplied by 6.25 to obtain protein content in the sample (McClements, 2003).

5.2.2.2 Dietary fibre

Dietary fibre content determination consisted of removing all digestible substances of the samples and weighing the residue. After fat extraction, one (1) gram of each sample was used to assess dietary fibre content. Each sample was dissolved in 50 ml of phosphate (pH 6), 0.1 ml of amylase was added and the solution was incubated at 95°C for 15 minutes. After the incubation, the solution was cooled to room temperature and its pH was adjusted to 7.5 by adding NaOH (0.275 N). 0.1 ml of protease was added to the solution and placed in a water bath at 60°C for 30 minutes. At the end of this second incubation, the solution was cooled to room temperature and the pH adjusted between 4

and 4.6 by adding HCl (0.325M). 0.1 ml of Amyloglucosidase was added to the solution which was placed again in a water bath at 60°C during 30 minutes. By the end of this third incubation, 4 volumes of ethanol (95%) were added and the solution was let cool overnight at room temperature. After complete precipitation overnight, the solution was filtered and rinsed with ethanol (95%) and acetone to extract dietary fibre. After filtration, dietary fibre was dried in an oven at 70°C overnight and then weighed to obtain the fibre content (SIGMA, 2008).

5.2.2.3 Lipids

Lipid extraction was carried out using a 2050 SOXTEC Auto extraction unit (Foss, Denmark). 6 g of *A. zebrina* ground flower sample was weighed into a pre-weighed porous thimble which was hooked onto the extraction unit. 70 ml of petroleum spirit was poured into the thimble as a solvent and lipid was extracted by setting the unit at 135°C for 40 minutes. The thimble was transferred to the oven at 60°C for about 10 minutes to dry the sample and weighed to determine the lipid content (Foss, 2008).

5.2.2.4 Ash

Ash content was assessed by burning 2 grams of each *A. zebrina* flower sample in a furnace at 600°C for 12 hours. When samples are burnt, water and volatile substances are vaporized while organic substances are transformed into CO_2, H_2O and N_2 in the presence of oxygen. The sample was finally cooled and weighed to determine the ash content.

5.2.2.5 Vitamins

High Performance Liquid Chromatography (HPLC) was used to analyze Vitamin B complex: B1 (thiamine), B2 (riboflavin), B3 (nicotinic acid) and B6 (pyridoxine). These were analyzed using Varian Prostar 800 HPLC (Walnut Creek, CA, USA). A sample of 1.0524 g was weighed and ground in 10 ml of acetonitrile buffer (50 mM NaH2PO4 + 50 Mm H3PO4 + 0.8 mM Sodium 1- octaresulfnate pH 2.2) at 90:10 (0.8 ml min 1- : ml of buffer) at the column temperature of 40°C. The mixture was put in 2.0 µl reaction tubes, which were centrifuged at 14000 rpm for 5 minutes. The liquid mixture (leaving the lysate) was pipetted into a smaller bottle using a syringe and placed the lid onto a

smaller bottle. Then, the bottle was loaded to the machine for injection. Three ground *A. zebrina* flower samples were analyzed to improve the accuracy of the value determined.

5.2.2.6 Minerals

Sodium, Calcium and Potassium were determined using a Flame photometer (Model 410). Sample solutions were prepared by adding 5 ml (5000*ul*) of concentrated HCl acid to 1 g of ashed *A. zebrina* flower sample into each vial. 0.5 ml of the mixture from each vial was pipette into a labelled bottle, and then 9.5 ml of distilled water was added to the labelled bottle. Six standards (0, 5, 10, 25, 35 and 50 mg l^{-1}) were prepared in six 50 ml flasks. The standards and the sample solutions were read by the flame photometer. A regression equation was derived between standard solutions and the readings of the flame photometer and the equation was used to obtain the concentration of elements in sample solutions.

Phosphorus content was determined using the colorimetric method (Ames, 1966). This method is based on the principle that phosphate ions react with ammonium molybdate to give, when reduced by ascorbic acid, a blue complex which has an intense absorption band at 820 nm. The complex absorbance which is proportional to phosphate concentration in the original solution is then measured by a spectrophotometer (BioTek, model PowerWave XS). Sample solutions were prepared as described above. Six standard solutions (0, 10, 30, 50, 70 and 100 mg l^{-1}) were also prepared. 80 µl of sample and standard solutions were placed in a 96 well plate, 180 µl of Ames reagent was added at 30 second intervals and finally 30 µl of ascorbic acid (10 %) was added in each well plate. The absorbance of the solutions was read by the spectrophotometer after 15 minutes at an interval of 30 seconds. The regression equation between the concentration of standard solutions and the readings of the spectrophotometer was used to obtain the phosphate concentration in sample solutions.

Magnesium, Manganese, Iron and Zinc were determined using Atomic Absorption Spectrophotometer (Varian Spectr AA 220 FS). The principle is that each element when burnt emits a specific wavelength light which is proportional to the content of the element in the solution. Six concentrations of each element were used as standards to

calibrate the photometer. Sample solutions were prepared as described above. The absorbance of the sample and standard solutions read by the atomic absorption photometer was used to determine the element concentration.

5.3 Results

Composition, vitamin and mineral content analyses of *A. zebrina* flowers are shown in Tables 5.1, 5.2 and 5.3, respectively.

Table 5.1 Proximate composition of *A. zebrina* flowers of Namibia

Proximate component	**(%±SE)**
Protein	14.2±0.13
Ash	5.3±0.13
Fat	1.5±0.21
Dietary fibre	50.5±1.17

Table 5.2 Vitamin content of *A. zebrina* flowers of Namibia

Vitamin	**Composition (mg / 100g±SE)**
Nicotinic acid (B3)	5.4±0.60
Pyridoxine (B6)	2.6±0.81
Thiamine (B1)	1.0±0.40
Riboflavin (B2)	None detected

Table 5.3 Mineral analysis of *A. zebrina* flowers in Namibia

Mineral	(g /100g±SE)
Calcium	6.0±0.19
Potassium	86.1±9.91
Magnesium	26.0±0.47
Phosphorus	0.1±0.00
Sodium	11.4±1.33
Iron	3.2±0.36
Manganese	0.2±0.01
Zinc	0.6±0.03

5.4 Discussion

Protein: Based on the results of the current study, a 100 g of *A. zebrina* flower product can provide 14.2 g of protein which is about ¼ and ⅓ of men and women protein daily requirements, respectively (Table 5.4). Only 22% of foods presented in Appendix III have protein content higher than *A. zebrina* flower products (>14 g per 100 g). That means, 78% of foods listed in Appendix III had less protein content in comparison to *A. zebrina* flower (<14 g per 100 g). This indicates that *A. zebrina* flower products are rich in protein compared to most foods (Appendix III).

Table 5.4 Daily dietary intake requirement of food nutrients (g) for humans

Nutrient	Adult male	Adult female	Both
Protein	56 g	46 g	
Dietary fibre	30-38 g	21-25 g	
Vitamin B1			0.90 – 1.20 mg
Vitamin B3			12 - 16 mg
Vitamin B6			1.0 - 1.5 mg
Ca		1.0 - 1.6 g	
K		4.5 – 4.7 g	
Mg	0.4 g	0.3 g	
P			3 – 4 g
Na			2.3 g
Fe			0.01 g
Mn			0.01 g
Zn			0.01 g

Sources: IOM (2001), Roizen (1997), Ward (2008)

Dietary fibre: According to the results of the present study, a 100 g flower of *A. zebrina* can provide 54.5 g of dietary fibre which is almost twice the daily recommended amount (Table 5.4). Relating these findings to other vegetables in previous studies, Gebhardt (2002) reported dietary fibre content of cooked cabbage as 3.5 g and raw lettuce as 1.6 g. This shows that *A. zebrina* flower product is very rich in dietary fibre.

Ash: Nielsen (1998) documented that ash contents of fresh foods rarely exceed 5%, although some processed foods, for example dried beef, can have as high as 12% ash contents. The amount of ash (5.3%) found in *A. zebrina* flower product is within the range reported in literature, but is low compared to findings of the study conducted by Bear *et.al* (1948) where ash content in cabbage was 8.2%, lettuce 11.5% and spinach 26.1%.

Vitamins: The value obtained for nicotinic acid of 5.39 mg per 100 g in *A. zebrina* dried flower product was less than half of daily recommended intake of 12 - 16 mg; but 2.63 mg the corresponding level for pyridoxine per 100 g found was higher than the daily recommended intake of 1.00 - 1.50 mg, and higher than the concentration of pyridoxine in sweet potato leaves of 0.2 g (Woolfe, 1992). The value for thiamine of 0.95 mg is well within the range of daily recommended intake of 0.90 – 1.20 mg (IOM, 1998). This means that 100 g of *A. zebrina* flower product can provide the recommended daily amount of pyridoxine as well as thiamine. *A. zebrina* flower have a large amount of these vitamins compared to the values reported by Gebhardt (2002) of and nicotinic vitamins for cooked cabbage and lettuce (0.09 mg thiamine and 0.40 mg nicotinic acid) and (0.10 mg thiamine and 0.50 mg nicotinic acid), respectively.

Fat: The fat content of 1.5 g per 100 g of *A. zebrina* flower product is well within the range of composition values reported for most food items (Appendix III) and is comparable to the fat content of 100 g for boiled brown rice of 1.4 g and that of white bread of 1.6 g.

Calcium: The Ca content of *A. zebrina* of 6 g per 100 g shows that 100 g of flower product can provide 5 days of Ca requirement for one person (Table 5.4). The values

obtained were also higher compared to other vegetables such as cabbage (0.7 g), lettuce (0.7 g) and spinach (1.4 g) (Bear *et al.*1948).

Potassium: The content of K of 86.1 g per 100 g found in *A. zebrina* flower was very high. This means a 100 g of *A. zebrina* flower can provide the daily potassium requirement for 19 people (Table 5.4). The amount of K found was also higher compared to several other crops reported by Bear *et al.* (1948) such as cabbage (2.7 g), lettuce (4.2 g) and spinach (7.4 g).

Magnesium: The amount of Mg of 26 g per 100 g found in *A. zebrina* flower was very much higher than the values reported for most food items shown in Table 5.5. This means a 100 g of *A. zebrina* flower could provide the daily Mg requirement of 65 people (Table 5.4).

Table 5.5 Magnesium content of some common foods

Food type	Food content (mg)	Mg content (mg)
Almonds	680 260	119
Artichoke hearts	1 360 520	50
Baby Lima beans	1 360 520	46
Medium baked potato with skin	3800	55
Medium banana	3000	34
Black beans	1 360 520	60
Brown rice, cooked	1 360 520	42
Butternut squash	1 360 520	36
Chicken breast	85 050	25
Green peas, cooked	1 360 520	23
Green soybeans	1 360 520	83
Cashews	1 360 520	157
Halibut, baked	1 360 520	78
Low-fat yogurt	2 721 040	43
Milk, none fat	2 721 040	28

Navy beans	1 360 520	52
Oatmeal, cooked	2 721 040	56
Peanuts	1 360 520	125
Peanut Butter	28 350	54
Pumpkin seeds	680 260	303
Soy nuts	1 360 520	196
Spinach, cooked	1 360 520	66
Sunflower seeds	680 260	82
Swiss Chard, cooked	1 360 520	75
Tofu, firm	1 360 520	128
Unsweetened chocolate	28 350	88
Wheat Germ	28 350	45

Table adapted from: Hands, E.S. (2000)

Keys: ¼ cup = 680 260 mg; ½ cup = 1 360 520 mg; 1 cup = 2 721 040 mg; 1 oz = 28 350 mg; 3 oz = 85 050 mg; 2 teaspoons ≈ ¼ cup = 680 260 mg (Unruh and Brecke, 1993).

Phosphorus: The amount of P of 0.1g per 100 g of *A. zebrina* flower product was very low, but comparable with P content of other vegetables namely cabbage (0.3 g), lettuce (0.3 g) and spinach (0.3 g) (Bear *et al.*1948). 100 g of *A. zebrina* flower can provide 4% of P required per day (Table 5.4).

Sodium: Sodium content of 11.4 g per 100 g of *A. zebrina* flower product can provide the Na requirement for one person for 5 days (Table 5.4). The value was also high compared to the sodium content in other crops such as cabbage (0.1 g), lettuce (0.1 g) and spinach (0.6 g) (Bear *et al.* 1948).

Iron: Gebhardt (2002) reported Fe content in cooked cabbage and lettuce was close to zero, that is, 0.30 mg per 100 g and 0.50 mg per 100 g, respectively. The quantity of Fe obtained from *A. zebrina* flower product was 3.20 g per 100 g, which was dramatically higher. 100 g of *A. zebrina* flower product could provide the daily Fe requirement for 318 people (Table 5.4).

Manganese: The value of Mn of 0.2 g per 100 g obtained from *A. zebrina* flower product was higher than the daily Mn requirement (Table 5.4). 100 g of *A. zebrina* flower product could provide the daily requirement for 15 people. However, the value was lower than that of tomatoes (4.5 g) (Bear *et al*, 1948).

Zinc: The value of Zn (0.6 g per 100 g) obtained from *A. zebrina* flower product was higher than the amount of Zn required per day (Table 5.4). 100 g of *A. zebrina* flower product could provide the daily Zn requirement for 56 people. The value is also similar to value of 0.5 g reported by French (2006) for raw stalks of Asparagus and boiled leaves of beetroot.

Since average weight of *A. zebrina* cake is about 52 g, which could be normally enough for a meal of about 5 people. That means one cake would provide the following nutrients: 7.4 g of protein, 28.3 g dietary fibre, 2.7% ash, vitamins (2.80 mg nicotinic acid, 1.36 mg pyridoxine, 0.49 mg thiamine), 0.8 g fat, 3.1 g calcium, 44.8 g potassium, 13.5 g magnesium, 0.05 g phosphorus, 5.9 g sodium, 0.16 mg iron, 0.1 g manganese and 0.3 g of zinc.

CHAPTER 6

GENETIC DIVERSITY IN *ALOE ZEBRINA* POPULATIONS IN NAMIBIA

This chapter estimates genetic diversity in natural populations of *Aloe zebrina*. The chapter is divided into five sections, subdivided in subsections as appropriate. The first section addresses an introductory to the current research, composed into general background, genomic size and AFLP molecular markers technique. Secondly, research objectives were presented, thirdly methodology applied during this study which was constituted into sampling of *A. zebrina* populations for genetic material collection, DNA extraction, DNA quantification, AFLP marker production, PCR samples optimization, separation and scoring of AFLP markers as well as how genetic diversity data were analyzed. Presentation of results was based onto the level of polymorphism and genetic diversity obtained, while the last section discusses the outcome of this study.

6.1 Introduction

6.1.1 General background

Knowledge of population structure and genetic diversity is a prerequisite for development of conservation strategies and for domestication of germplasm resources (Mwase *et al.* 2006). The genus *Aloe* and its close relatives are reported as being remarkable for the stability in the number and size of chromosomes (Reynolds, 2004). Despite the widespread occurrence of *A. zebrina* in Southern Africa, it is protected against collection and trade from the wild by CITES regulations as are other *Aloe* species (PROTA, 2006). Although it is recognized as an important resource for food, medicine, feed for animals and for its ornamental value, little is known about its genetic variation. The current study was therefore conducted in an attempt to determine the genetic diversity of *A. zebrina* within and between populations in Otjozondjupa, Ohangwena and Omusati regions of Namibia.

6.1.2 Genomic size

It has been documented that every species of *Aloe* that has been investigated cytologically was found to have a single basic chromosome number (x = 7) and a large, strongly bimodal karyotype, always comprising three short chromosomes and four much longer ones in the haploid set. Where a considerable interspecific variation in overall chromosome length has been reported, as reflected in the nuclear DNA C-value, which ranges in diploid (2n=14) species from 4C=41.78 picograms (pg) in *A. tenuior* Haw. - 95.4 pg in *A. peckii*, same as documented by Adams (2000) as 1DNA=10.5 pg to 23.9 pg, respectively. *A. zebrina* was reported to be one of advanced *Aloe* species with 4C=79.63 pg (Reynolds, 2004; Adams, 2000).

The majority of *Aloe* species including *A. zebrina* (*A. ammophila*) are diploid (2n=14) but other ploidy has been reported in the genus, such as triploidy (2n=21), tetraploidy (2n=28) and hexaploidy (2n=42) (Reynolds, 2004; Brandham and Doherty, 1998; Brandham, 2004).

According to Brandham & Doherty (1998) there was a strong indication that primitive species, for example *A. tenuior*, had the lowest nuclear DNA amount, which means smallest chromosome set although the number of species examined was not large (*A. zebrina* was included). Further, the amount of DNA and overall chromosome size was noticed to increase with evolutionary advancement. Therefore, in *Aloe*, karyotype is uniform but genomic DNA amounts vary.

6.1.3 AFLP molecular markers technique

Molecular markers are commonly used to characterize genetic diversity within and between populations or groups of individuals because they typically detect high levels of polymorphism. Amplified Fragment Length Polymorphism (AFLP) is a novel Polymerase Chain Reaction (PCR) based technique for DNA fingerprinting that relies on PCR amplification of a subset of small restriction fragments. AFLP is reported as being efficient in allowing multiple loci to be analysed for each

individual in a single gel run (Kosman and Leonard, 2005). According to Zhivotovsky (1999), AFLP is usually chosen because of its capacity to survey a much larger number of loci for polymorphism than other currently available PCR-based methods such as Simple Sequence Repeats (SSR) or Microsatellites, Random Amplified Polymorphic DNA (RAPD) and Single Nucleotides Polymorphism (SNPs).

Molecular markers are polymorphic when there is DNA sequence variation between the individuals under study and polymorphisms are detected as the presence or absence of an amplified restriction fragment. They are simply an indicator of sequence polymorphism, and can take many forms between individuals, ranging from insertion or deletion of multiple bases, down to single nucleotide polymorphisms (SNPs) (Henry, 2001).

6.2 Research objectives

The present research was carried out to determine the genetic diversity within and between *A. zebrina* populations in the three study regions of Namibia by using AFLP molecular marker technique.

The main null hypothesis to be tested under this experiment is that there is no variation within or between *A. zebrina* populations in Otjozondjupa, Ohangwena and Omusati regions.

6.3 Materials and methods

6.3.1 Sampling of *A. zebrina* populations for genetic material collection

Plant samples of *A. zebrina* were collected from all the 8 study sites (Figure 1.1). Three plants per plot from each study site (making a total of 84 plants) were collected. The plants were then transplanted into polythene pots using soils collected under *Acacia erioloba* trees at Ogongo Agricultural College Forestry Nursery. The 84 potted *A. zebrina* plants were transported to the greenhouse of the University of Namibia (main campus) in Windhoek (the capital city of the Republic of Namibia) for extraction of the plant's genomic DNA. One individual *A. vera* plant (used as an out-group) was obtained from Llanfairpwllgwyngyllgogerychwyrndrobwllllantysiliogogogoch in Angelsey, North Wales, United Kingdom (John Gorham's personal plant collection).

6.3.2 DNA extraction

A single DNA sample was extracted from each of the 84 individual *A. zebrina* plants in the plant molecular biology laboratory, Department of Science at the University of Namibia (UNAM). Extracted DNA samples were transported from the University of Namibia to Bangor University in the United Kingdom for AFLP assay. DNA extraction for *A. vera* (used as an out-group), was carried out in the molecular biology laboratory of the School of Biological Sciences (SBS) at Bangor University (BU). Four genomic DNA samples were extracted from a single *A. vera* plant and were used as an out group during DNA sample analysis.

DNA was extracted from 100 mg of fresh leaf tissue using the QIAGEN DNeasy extraction kit. Samples were ground using a pestle and mortar and a spatula was used for scooping ground samples. Liquid nitrogen was used for chilling leaf tissues. The liquid nitrogen was purchased from Afrox gases Company (every day immediately just before DNA extraction), transported to UNAM in a thermo-flask, kept frozen and the DNA was extracted without delay. For *A. vera* samples, liquid nitrogen was collected from Chemistry department, Bangor University. The DNeasy protocol is detailed in Appendix IV.

6.3.3 DNA quantification

Two DNA quantifications were carried out, the first check was conducted at the University of Namibia immediately after each extraction by preparing and running a 1% agarose mini gel (run at 60 V for 35 minutes) in 1 x TAE buffer containing 2.5µl of ethidium bromide. A marker (200bp ladder) was run alongside DNA samples to quantify them, DNA bands were then visualized on an UVitec gel documentation system and results recorded. The mini gel protocol is detailed in Appendix V

The second DNA quantification was done at Bangor University (after transportation of extracted DNA samples from Namibia to the UK), in order to re-check DNA concentration of samples before AFLP assay. 1% agarose mini gel was prepared and run at 50 V for 15 minutes in 1 x TBE buffer containing 2.5µl of ethidium bromide. Five Lambda DNA size standards (6.25; 12.5; 25; 50 and 100 ng/µl) were run alongside the samples to quantify extracted DNA samples after transportation from Namibia to the UK. The genomic DNA was visualized and photographed under ultra-violet light source. Some DNA degradation was observed after transporting DNA samples to the UK. The mini gel protocol is detailed in Appendix VI. The amount of DNA in each sample was then estimated referring to the five size standards and the results were compiled as shown in Table 6.1.

Table 6.1 Quantification of genomic DNA in *A. zebrina* and *A. vera* samples using mini gels with comparison against five Lambda DNA size standards.

Sample number	Site and plot number	Estimated DNA concentration (ng/µl)	Total number of sample with estimated DNA concentration / site
1	S1P1p1	25	
2	S1P1p2	25	
3r	S1P1p3	50	
4	S1P2p1	50	
5	S1P2p2	0	
6	S1P2p3	100	
7	S1P3p1	0	
8r	S1P3p2	25	
9	S1P3p3	0	
10	S1P4p1	0	
11r	S1P4p2	100	
12	S1P4p3	0	
13	S1P5p1	50	
14r	S1P5p2	100	
15	S1P5p3	0	9
16	S2P1p1	25	
17r	S2P1p2	50	
18	S2P1p3	0	
19	S2P2p1	25	
20	S2P2p2	0	
21	S2P2p3	25	
22	S2P3p1	0	
23	S2P3p2	0	
24	S2P3p3	50	
25	S2P4p1	0	
26	S2P4p2	0	
27	S2P4p3	0	
28	S2P5p1	0	
29r	S2P5p2	100	
30	S2P5p3	25	7
31	S3P1p1	25	
32	S3P1p2	50	
33	S3P1p3	100	
34	S3P2p1	25	
35	S3P2p2	50	
36r	S3P2p3	100	
37	S3P3p1	50	
38r	S3P3P2	50	
39	S3P3p3	0	8

40	S4P1p1	0	
41r	S4P1p2	100	
42r	S4P1p3	100	
43	S4P2p1	50	
44	S4P2p2	50	
45	S4P2p3	50	
46	S4P3p1	25	
47	S4P3p2	100	
48	S4P3p3	50	8
49	S5P1p1	12.5	
50r	S5P1p2	50	
51	S5P1p3	0	
52	S5P2p1	0	
53	S5P2p2	25	
54	S5P2p3	0	
55	S5P3p1	12.5	
56	S5P3p2	0	
57	S5P3p3	0	4
58	S6P1p1	0	
59	S6P1p2	0	
60	S6P1p3	0	
61	S6P2p1	0	
62	S6P2p2	50	
63	S6P2p3	50	
64	S6P3p1	0	
65r	S6P3p2	100	
66	S6P3p3	50	4
67	S7P1p1	25	
68r	S7P1p2	50	
69	S7P1p3	0	
70	S7P2p1	0	
71	S7P2p2	100	
72	S7P2p3	50	
73	S7P3p1	25	
74r	S7P3p2	100	
75	S7P3p3	100	7
76	S8P1p1	25	
77	S8P1p2	0	
78	S8P1p3	0	
79	S8P2p1	0	
80	S8P2p2	25	
81	S8P2p3	50	
82	S8P3p1	50	
83	S8P3p2	100	
84	S8P3p3	100	6
Aloe zebrina			53

Aloe vera (out-group)			4
Total potential samples			57

Keys: r = repeated DNA extraction sample, S = site, P = plot number, p = plant number

On the basis of the results shown in Table 6.1, it was decided to carry out the AFLP analysis on those samples where DNA concentrations were above 12.5 ng/µl (53 samples). Those below this value was considered degraded and were not analyzed further.

6.3.4 AFLP markers production

Generation of AFLP markers was carried out following the Vos *et al.* (1995) protocol adapted for the Beckman Coulter Sequencer CEQ 8000. Detailed AFLP protocol is presented in Appendix VII. DNA samples were prepared for restriction digestion based on the DNA concentration of each sample as shown in Appendix VIII.

Production of AFLP markers was carried out in four steps. Firstly, the process was started with digestion of *A. zebrina* genomic DNA with two different restriction enzymes *Eco*RI and M*Se*I (*Tru*9I). Restriction enzymes are responsible for cutting genomic DNA at a specific recognized sequence as shown in Table 6.2, which generate hundreds of thousands of DNA fragments. The two restriction enzymes produce different "sticky" ends to which adapters are ligated.

Table 6.2 Recognition sequence of restriction enzymes used in the present investigation to digest *A. zebrina* genomic DNA.

Restriction enzymes	Recognition site
*Eco*RI	5'...G˅AATTC...3' 3'...CTTAA˄G...5'
Mse (*Tru*9I)	5'...T˅TAA...3' 3'...AAT˄T...5'

Secondly, double-stranded adapters (as illustrated in figure 6.1) complementary to the cut ends (overhangs) produced by *Eco*RI and *MSe*I (*Tru*9I) enzymes were ligated to the cut DNA fragments. This resulted in DNA fragments having three different adapter combinations at their ends, namely *Eco*RI – *Eco*RI, *Eco*RI - *MSe*I and *Mse*I - *Mse*I.

Thirdly, the first PCR amplification (pre-amplification / pre-selective) including two oligonucleotides (primers) corresponding to the sequence of the adaptor was carried out using universal primers E00 and M00 as shown in table 6.3. The thermocycler conditions includes 30 cycles consisting of 30 seconds denaturation at 90^{0}C, 60 seconds annealing at 56 ^{0}C, 60 seconds extension at 72 ^{0}C and finally 600 seconds extension at 72 ^{0}C. The pre-selective PCR product, which is 5µl of each PCR reaction, was run on a mini agarose gel together with a 1 kb ladder to check the success of amplification and the size range of products generated. This is important for quality control (Bensch and Åkesson, 2005).

Table 6.3 Nucleotide sequences of the adaptors and primers used in the ligation and pre-selective amplification stages of the AFLP process in *A. zebrina*.

Adaptor/Primer	Adaptor/Primer sequence
*Eco*RI adaptor	5'-CTC GTA GAC TGC GTA CC-3' 3'-CAT CTG ACG CAT GGT TAA-5'
*Mse*I adaptor	5'-GAC GAT GAG TCC TGA G-3' 3'-TA CTC AGG ACT CAT-5'
E00 pre-selective primer	5'-GTA GAC TGC GTA CCA ATT C-3' 3'-C TTA ACC ATG CGT CAG ATG-5'
M00 pre-selective primer	5'-GAC GAT GAG TCC TGA GTA A-3' 3'-A ATG AGT CCT GAG TAG CAG-5'

Finally a second PCR amplification (selective amplification) was carried out using selective primers (E00 + 3; M00 + 3), each with three nucleotide extensions. Selective primer (E00 + 3) was labeled with fluorescent dye D4, meanwhile selective primer (M00 + 3) was unlabeled. The thermo cycler conditions included 13 touchdown cycles where the annealing temperature was reduced gradually to avoid amplifying non-specific sequences (30 seconds denaturation at 94 ^{0}C, 30 seconds annealing at 65 ^{0}C, which was

then reduced by 0.7 ^{0}C per cycle, 60 seconds extension at 72 ^{0}C), 23 normal cycles (30 seconds denaturation at 94 ^{0}C, 30 seconds annealing at 56 ^{0}C, 60 seconds extension at 72 ^{0}C) and a final extension on hold at 10^{0}C. Pre-amplification as well as selective amplification were carried out one after the other using the thermo cycler (MJ Researcher). All 84 *A. zebrina* DNA samples were analysed in a randomized order in relation to the groups and populations that were being compared in order to reduce methodological errors (Bensch and Åkesson, 2005).

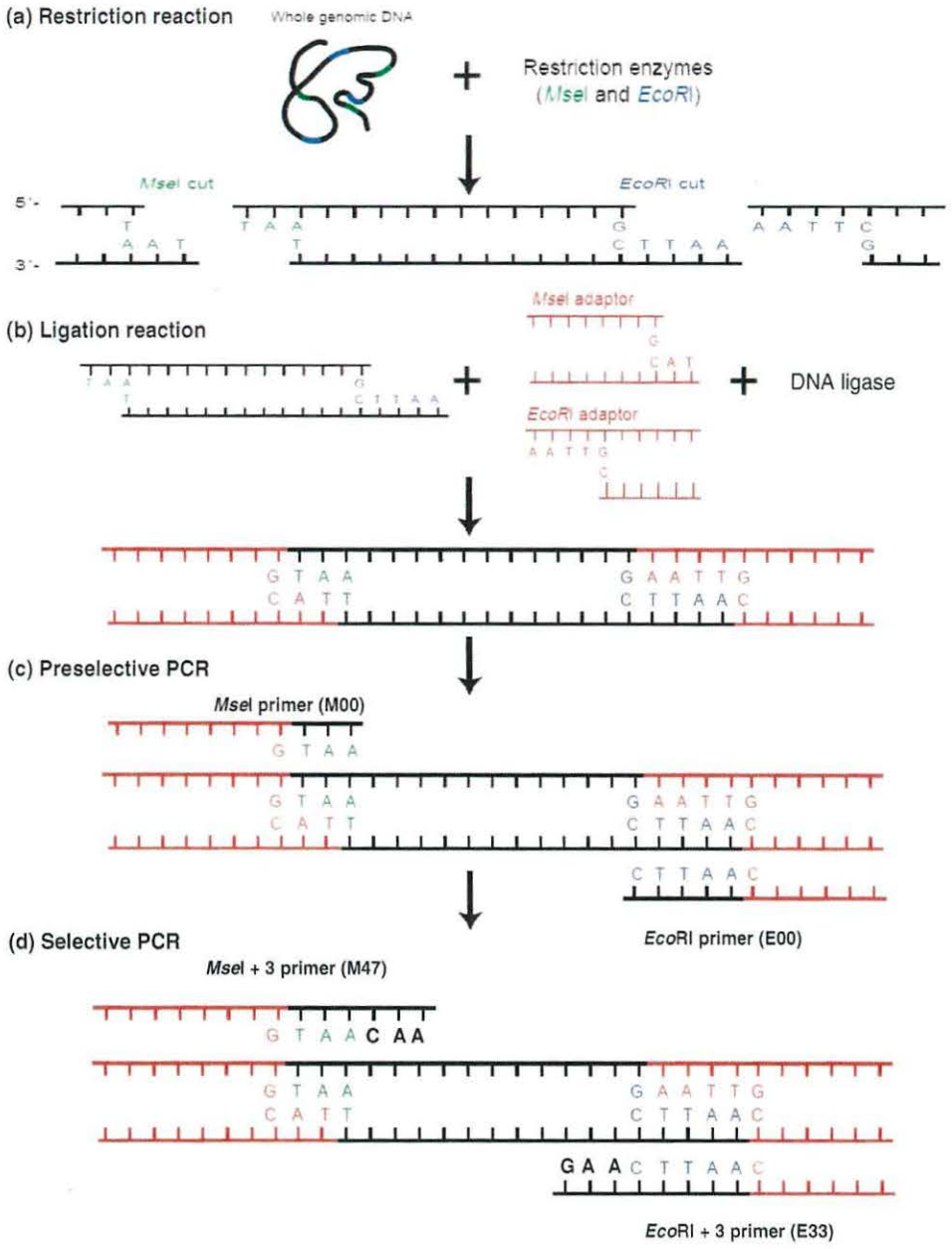
Eight selective primer pairs were used in the present experiment using all 84 individual samples to test for their ability to generate polymorphic loci. A polymorphic locus is when at least one peak is detected at a different location in any of the individuals included in the sample, while a monomorphic locus is when peaks are detected at the same locations in all individuals.

6.3.5 PCR samples optimization

PCR product samples were optimized to obtain the best possible sample trace (peaks between 1000 and 100 000 relative fluorescence units). Selective amplification PCR products were diluted in Sample Loading Solution (de-ionized formamide from Beckman Coulter) to the ratio of 1:2. Samples were prepared by pipeting 5 µl of SLS and mixed it with 2.5 µl of selective amplification PCR products.0.5 µl of the diluted selective amplification PCR products were loaded for fragment analysis.

The second optimization experiment used 0.5 µl of undiluted selective amplification PCR products for fragment analysis. This worked better than using diluted samples, so undiluted selective amplified PCR products were used during this study. PCR product optimization protocol is detailed in Appendix VIIII.

Reproducibility of AFLP peaks was checked by repeating the whole AFLP process (Appendix VII), starting from digestion of genomic DNA to fragment analysis stage with four individual *A. zebrina* samples selected at random.



Adapted from Mueller and Wolfenbarger (1999)

Figure 6.1 Diagram representing the steps involved in the generation of AFLP markers in *Aloe zebrina*. (a) digestion of genomic DNA with two restriction enzymes, (b) ligation of adapters to the digested DNA, (C) pre-selective amplification with universal primers, (d) selective amplification with selective primers.

6.3.6 Separation and scoring of AFLP markers

The selective amplified PCR products were separated through capillary gel electrophoresis in a CEQ 8000 Genetic Analysis System (Beckman Coulter Inc.) and analyzed with the Fragment analysis software. During Fragment analysis the separated fragments were sized by using internal size standards (PA400). Following this, the sized fragments were subjected to an AFLP binning analysis that converted the AFLP peak profiles into binary matrix composed of ones and zeroes at each locus as reported by Mishra *et al.* (2009). Only the peaks between 1000 and 100 000 rfu (relative fluorescence unit) were considered in the binning analysis. The presence of a peak was scored 1 and its absence was scored 0 as documented by (Lynch and Milligan, 1994). Peaks having same length detected in different individuals were placed together in bins with maximum bin width of 1.0. Peaks of size ranging from 80bp to 380bp were scored.

6.3.7 Genetic diversity data analysis

The NTSYSpc 2.10t software package was used to do cluster analysis, and to compute pairwise Jaccard's genetic distance estimates and the Jaccard coefficient of similarity using operational taxonomic units (OTUs) from AFLP markers. A dendrogram was generated by using Unweighted Pair Group method with arithmetic Mean (UPGMA) method.

6.4 Results

6.4.1 Level of polymorphism

The eight primer combinations generated a total of 359 AFLP bands, out of which 309 were Polymorphic, representing 86% Polymorphism (heterozygosity) and only 14% (50) were monomorphic from all the 52 operational taxonomic units (OTUs). The total number of Polymorphic bands scored per primer pair ranged from 12 (E-AAC/T-CAT) to 77 (E-AAG/M-CAA) with an average of 39 bands per primer combination, which suggests a high degree of variability between individual plants of *A. zebrina* as shown in Table 6.4.

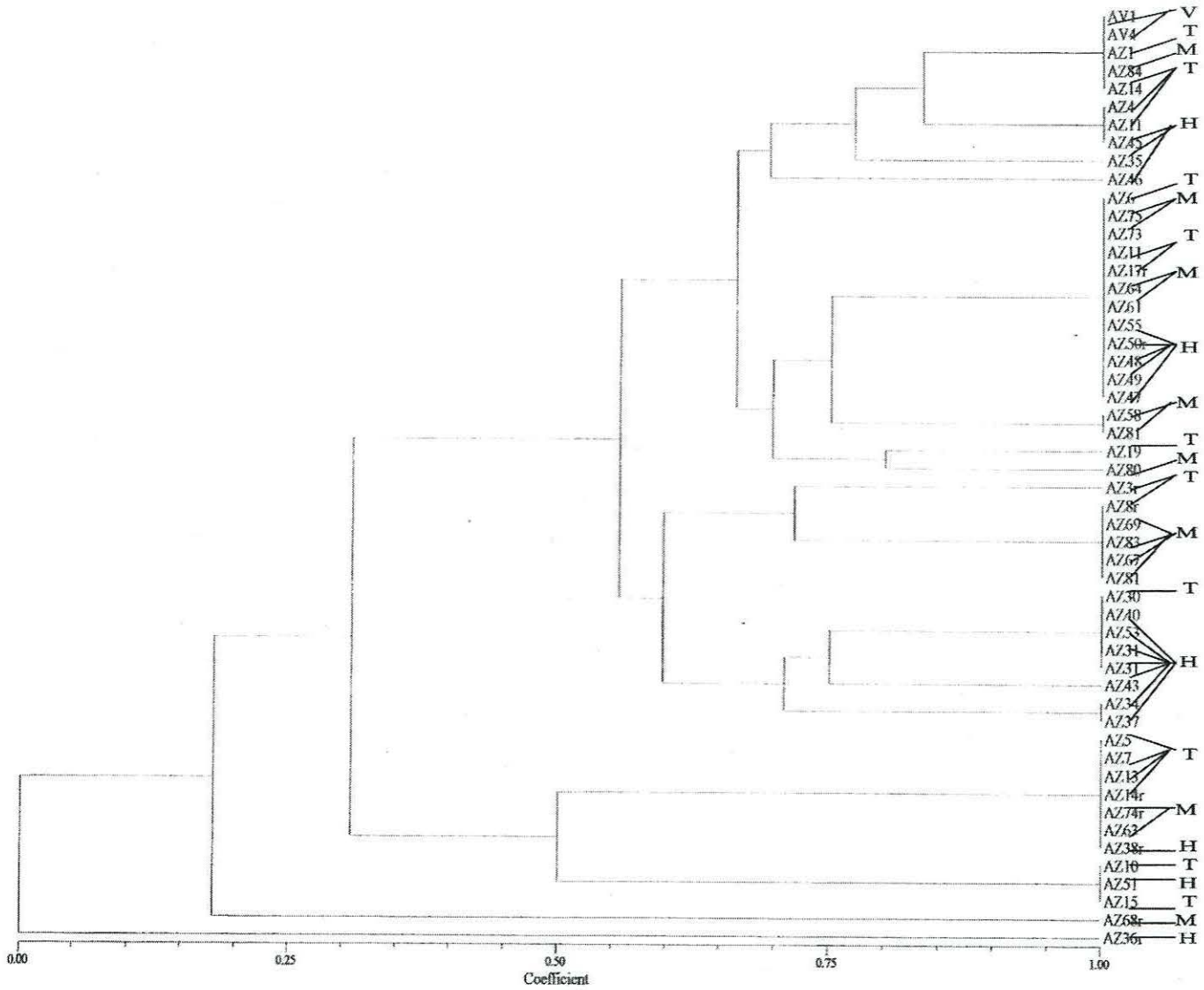
Table 6.4 Level of polymorphism and fingerprinting of AFLP markers in *A. zebrina* (N=52).

Primer name	Primer combinations	Total bands	Polymorphic bands	Polymorphic rate %
E32M50	*Eco*RI-AAC / *Tru*9I-CAT	12	12	100
E32M47	*Eco*RI-AAC / *Tru*9I-CAA	15	15	100
E32M62	*Eco*RI-AAC / *Tru*9I-CTT	17	17	100
E33M50	*Eco*RI-AAG / *Tru*9I-CAT	26	26	100
E33M62	*Eco*RI-AAG / *Tru*9I-CTT	30	27	90
E35M50	*Eco*RI-ACA / *Tru*9I-CAT	109	62	57
E35M47	*Eco*RI-ACA / *Tru*9I-CAA	73	73	100
E33M47	*Eco*RI-AAG / *Tru*9I-CAA	77	77	100
	Total	359	309	86
	Mean	45	39	

6.4.2 Genetic diversity

Contrary to the high Polymorphism obtained above, no consistent pattern of variability was observed according to the cluster analysis carried out on the dataset. A dendrogram generated from AFLP results showed mean Jaccard coefficient of 0.50, which indicated symmetrical similarity or dissimilarity of 50% within and between *A. zebrina* populations in Otjozondjupa, Ohangwena and Omusati regions. No clustering of OTUs was observed either based on study sites or regions as shown in Figure 6.2.

Pairwise Jaccard's genetic distance estimates among OTUs (Table 6.5) ranged from 0.0 to 1.0. Mean pairwise Jaccard's distance coefficient between *A. zebrina* populations in Otjozondjupa, Ohangwena and Omusati regions was 0.27 (genetic difference), which suggests a 0.73 genetic closeness between populations.



Keys: AV = *Aloe vera*, AZ = *Aloe zebrina*, T= Otjozondjupa, H= Ohangwena, M= Omusati, r = Repeated DNA sample, 1-84 = sample number

Figure 6.2 A dendrogram showing similarity between *A. zebrina* populations in Otjozondjupa, Ohangwena and Omusati regions of Namibia.

Table 6.5 Half-matrix of pairwise Jaccard's distance coefficients between *A. zebrina* populations in three study regions in Namibia

	V1	V4	T1	T3	T4	T5	T6	T7	T8	T11	T13	T14	T17	T19	T10	T15	T30	H31	H34	H35	H36	H37	H38	H40	H43	H45	H46	H47	H48	H49	H50	H51	H53	H55	M58	M61	M63	M64	M67	M68	M69	M73	M74	M75	M80	M81	M83	M84
V1	1.0																																																
V4	1.0	1.0																																														
T1	1.0	1.0	1.0																																													
T3	0.7	0.7	0.7	1.0																																												
T4	0.8	0.8	0.8	0.5	1.0																																											
T5	0.2	0.2	0.2	0.3	0.2	1.0																																										
T6	0.7	0.7	0.7	0.6	0.8	0.3	1.0																																									
T7	0.2	0.2	0.2	0.3	0.2	0.0	0.3	1.0																																								
T8	0.4	0.4	0.4	0.7	0.5	0.0	0.7	0.0	1.0																																								
T11	0.7	0.7	0.7	0.6	0.8	0.3	0.0	0.3	0.7	1.0																																							
T13	0.2	0.2	0.2	0.3	0.2	0.0	0.3	0.0	0.0	0.3	1.0																																						
T14	0.2	0.2	0.2	0.3	0.2	0.0	0.3	0.0	0.0	0.3	0.0	1.0																																					
T17	0.7	0.7	0.7	0.6	0.8	0.3	0.0	0.3	0.7	0.0	0.3	0.3	1.0																																				
T19	0.8	0.8	0.8	0.8	0.7	0.2	0.8	0.2	0.5	0.8	0.2	0.2	0.8	1.0																																			
T10	0.3	0.3	0.3	0.5	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.4	1.0																																		
T15	0.3	0.3	0.3	0.5	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.0	1.0																																	
T30	0.7	0.7	0.7	0.6	0.8	0.3	0.6	0.3	0.7	0.6	0.3	0.3	0.6	0.5	0.5	0.5	1.0																																	
H31																																																	
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H31	0.7	0.7	0.7	0.6	0.8	0.3	0.6	0.3	0.7	0.6	0.3	0.3	0.6	0.5	0.5	0.5	1.0	1.0																			
H34	0.5	0.5	0.5	0.4	0.6	0.3	0.4	0.3	0.3	0.4	0.3	0.3	0.4	0.3	0.3	0.3	0.8	0.8	1.0																		
H35	0.8	0.8	0.8	0.5	0.7	0.2	0.5	0.2	0.2	0.5	0.2	0.2	0.5	0.7	0.2	0.2	0.5	0.5	0.6	1.0																	
H36	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0																
H37	0.5	0.5	0.5	0.4	0.6	0.3	0.4	0.3	0.3	0.4	0.3	0.3	0.4	0.3	0.3	0.3	0.8	0.8	1.0	0.6	0.0	1.0															
H38	0.2	0.2	0.2	0.3	0.2	1.0	0.3	1.0	0.0	0.3	1.0	1.0	0.3	0.2	0.5	0.5	0.3	0.3	0.3	0.2	0.0	0.3	1.0														
H40	0.7	0.7	0.7	0.6	0.8	0.3	0.6	0.3	0.7	0.6	0.3	0.3	0.6	0.5	0.5	0.5	1.0	1.0	0.8	0.5	0.0	0.8	0.3	1.0													
H43	0.5	0.5	0.5	0.4	0.6	0.3	0.4	0.3	0.3	0.4	0.3	0.3	0.4	0.3	0.7	0.7	0.8	0.8	0.5	0.3	0.0	0.5	0.3	0.8	1.0												
H45	0.8	0.8	0.8	0.5	1.0	0.2	0.8	0.2	0.5	0.8	0.2	0.2	0.8	0.7	0.4	0.4	0.8	0.8	0.6	0.7	0.0	0.6	0.2	0.8	0.6	1.0											
H46	0.7	0.7	0.7	0.3	0.8	0.3	0.6	0.3	0.3	0.6	0.3	0.3	0.6	0.5	0.5	0.5	0.6	0.6	0.4	0.5	0.0	0.4	0.3	0.6	0.8	0.8	1.0										
H47	0.7	0.7	0.7	0.6	0.8	0.3	1.0	0.3	0.7	1.0	0.3	0.3	1.0	0.8	0.5	0.5	0.6	0.6	0.4	0.5	0.0	0.4	0.3	0.6	0.4	0.8	0.6	1.0									
H48	0.7	0.7	0.7	0.6	0.8	0.3	1.0	0.3	0.7	1.0	0.3	0.3	1.0	0.8	0.5	0.5	0.6	0.6	0.4	0.5	0.0	0.4	0.3	0.6	0.4	0.8	0.6	1.0	1.0								
H49	0.7	0.7	0.7	0.6	0.8	0.3	1.0	0.3	0.7	1.0	0.3	0.3	1.0	0.8	0.5	0.5	0.6	0.6	0.4	0.5	0.0	0.4	0.3	0.6	0.4	0.8	0.6	1.0	1.0	1.0							
H50	0.7	0.7	0.7	0.6	0.8	0.3	1.0	0.3	0.7	1.0	0.3	0.3	1.0	0.8	0.5	0.5	0.6	0.6	0.4	0.5	0.0	0.4	0.3	0.6	0.4	0.8	0.6	1.0	1.0	1.0	1.0						
H51	0.3	0.3	0.3	0.5	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.4	1.0	1.0	0.5	0.5	0.3	0.2	0.0	0.3	0.5	0.5	0.7	0.4	0.5	0.5	0.5	0.5	0.5	1.0					
H53	0.7	0.7	0.7	0.6	0.8	0.3	0.6	0.3	0.7	0.6	0.3	0.3	0.6	0.5	0.5	0.5	1.0	1.0	0.8	0.5	0.0	0.8	0.3	1.0	0.8	0.8	0.6	0.6	0.6	0.6	0.6	0.5	1.0				
H55	0.7	0.7	0.7	0.6	0.8	0.3	1.0	0.3	0.7	1.0	0.3	0.3	1.0	0.8	0.5	0.5	0.6	0.6	0.4	0.5	0.0	0.4	0.3	0.6	0.4	0.8	0.6	1.0	1.0	1.0	1.0	0.5	0.6	1.0			
M58	0.5	0.5	0.5	0.4	0.6	0.3	0.8	0.3	0.3	0.8	0.3	0.3	0.8	0.6	0.7	0.7	0.4	0.4	0.2	0.3	0.0	0.2	0.3	0.4	0.5	0.6	0.8	0.8	0.8	0.8	0.8	0.7	0.4	0.8	1.0		
M61	0.7	0.7	0.7	0.6	0.8	0.3	1.0	0.3	0.7	1.0	0.3	0.3	1.0	0.8	0.5	0.5	0.6	0.6	0.4	0.5	0.0	0.4	0.3	0.6	0.4	0.8	0.6	1.0	1.0	1.0	1.0	0.5	0.6	1.0	0.8	1.0	
M63	0.2	0.2	0.2	0.3	0.2	1.0	0.3	1.0	0.0	0.3	1.0	1.0	0.3	0.2	0.5	0.5	0.3	0.3	0.3	0.2	0.0	0.3	1.0	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.5	0.3	0.3	0.3	0.3	1.0

M64	0.7	0.7	0.7	0.6	0.8	0.3	1.0	0.3	0.7	1.0	0.3	0.3	1.0	0.8	0.5	0.5	0.6	0.6	0.4	0.5	0.0	0.4	0.3	0.6	0.4	0.8	0.6	1.0	1.0	1.0	1.0	0.5	0.6	1.0	0.8	1.0	0.3	1.0										
M67	0.5	0.5	0.5	0.8	0.6	0.3	0.8	0.3	1.0	0.8	0.3	0.3	0.8	0.6	0.7	0.7	0.8	0.8	0.5	0.3	0.0	0.5	0.3	0.8	0.5	0.6	0.4	0.8	0.8	0.8	0.8	0.7	0.8	0.8	0.5	0.8	0.3	0.8	1.0									
M68	0.2	0.2	0.2	0.3	0.2	0.0	0.3	0.0	0.5	0.3	0.0	0.0	0.3	0.2	0.0	0.0	0.3	0.3	0.3	0.2	0.0	0.3	0.0	0.3	0.0	0.2	0.0	0.3	0.3	0.3	0.3	0.0	0.3	0.3	0.0	0.3	0.0	0.3	0.3	1.0								
M69	0.5	0.5	0.5	0.8	0.6	0.3	0.8	0.3	1.0	0.8	0.3	0.3	0.8	0.6	0.7	0.7	0.8	0.8	0.5	0.3	0.0	0.5	0.3	0.8	0.5	0.6	0.4	0.8	0.8	0.8	0.8	0.7	0.8	0.8	0.5	0.8	0.3	0.8	1.0	0.3	1.0							
M73	0.7	0.7	0.7	0.6	0.8	0.3	1.0	0.3	0.7	1.0	0.3	0.3	1.0	0.8	0.5	0.5	0.6	0.6	0.4	0.5	0.0	0.4	0.3	0.6	0.4	0.8	0.6	1.0	1.0	1.0	1.0	0.5	0.6	1.0	0.8	1.0	0.3	1.0	0.8	0.3	0.8	1.0						
M74	0.2	0.2	0.2	0.3	0.2	1.0	0.3	1.0	0.0	0.3	1.0	1.0	0.3	0.2	0.5	0.5	0.3	0.3	0.3	0.2	0.0	0.3	1.0	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.5	0.3	0.3	0.3	0.3	1.0	0.3	0.3	0.0	0.3	0.3	1.0					
M75	0.7	0.7	0.7	0.6	0.8	0.3	1.0	0.3	0.7	1.0	0.3	0.3	1.0	0.8	0.5	0.5	0.6	0.6	0.4	0.5	0.0	0.4	0.3	0.6	0.4	0.8	0.6	1.0	1.0	1.0	1.0	0.5	0.6	1.0	0.8	1.0	0.3	1.0	0.8	0.3	0.8	1.0	0.3	1.0				
M80	0.7	0.7	0.7	0.6	0.5	0.3	0.6	0.3	0.3	0.6	0.3	0.3	0.6	0.8	0.5	0.5	0.3	0.3	0.2	0.5	0.0	0.2	0.3	0.3	0.4	0.5	0.6	0.6	0.6	0.6	0.6	0.5	0.3	0.6	0.8	0.6	0.3	0.6	0.4	0.0	0.4	0.6	0.3	0.6	1.0			
M81	0.5	0.5	0.5	0.8	0.6	0.3	0.8	0.3	1.0	0.8	0.3	0.3	0.8	0.6	0.7	0.7	0.8	0.8	0.5	0.3	0.0	0.5	0.3	0.8	0.5	0.6	0.4	0.8	0.8	0.8	0.8	0.7	0.8	0.8	0.5	0.8	0.3	0.8	1.0	0.3	1.0	0.8	0.3	0.8	0.4	1.0		
M83	0.4	0.4	0.4	0.7	0.5	0.0	0.7	0.0	1.0	0.7	0.0	0.0	0.7	0.5	0.5	0.5	0.7	0.7	0.3	0.2	0.0	0.3	0.0	0.7	0.3	0.5	0.3	0.7	0.7	0.7	0.7	0.5	0.7	0.7	0.3	0.7	0.0	0.7	1.0	0.5	1.0	0.7	0.0	0.7	0.3	1.0	1.0	
M84	1.0	1.0	1.0	0.7	0.8	0.2	0.7	0.2	0.4	0.7	0.2	0.2	0.7	0.8	0.3	0.3	0.7	0.7	0.5	0.8	0.0	0.5	0.2	0.7	0.5	0.8	0.7	0.7	0.7	0.7	0.7	0.3	0.7	0.7	0.5	0.7	0.2	0.7	0.5	0.2	0.5	0.7	0.2	0.7	0.7	0.5	0.4	1.0
Mn	**0.6**	**0.5**	**0.5**	**0.5**	**0.5**	**0.3**	**0.6**	**0.3**	**0.4**	**0.5**	**0.3**	**0.3**	**0.5**	**0.4**	**0.4**	**0.3**	**0.4**	**0.4**	**0.3**	**0.3**	**0.0**	**0.2**	**0.2**	**0.3**	**0.2**	**0.3**	**0.2**	**0.3**	**0.3**	**0.3**	**0.3**	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**	**0.1**	**0.2**	**0.1**	**0.1**	**0.1**	**0.1**	**0.0**	**0.1**	**0.0**	**0.1**	**0.0**	**0.0**

Keys: V= *Aloe vera*, T = Otjozondjupa, H = Ohangwena, M = Omusati, 1-84 = DNA sample number, Mn = Mean

6.5 Discussion

The results of a genetic diversity study based on the total number of bands (359) produced from eight combined primer pairs indicated an overall Polymorphism of 86% with an average of 39 loci per primer combinations. This suggests a high degree of diversity between individual *A. zebrina* plants in Namibia.

A dendrogram, however, showed no significant grouping of *A. zebrina* populations, neither by study sites nor by study regions. This means that individual samples collected from all three study regions were found spreading all over constructed dendrogram and no pronounced grouping was observed (Figure 6.2).

Since *A. zebrina* is found growing in clusters and reproduce by rhizomes, this may increase the possibility of inbreeding which is likely to decrease overall diversity. The mean pairwise Jaccard's distance coefficient between *A. zebrina* populations in the three study regions showed 0.27 (0.0 = completely different) and 0.73, (1.0 = homozygous). This is in agreement with what was reported by Kosman and Leonard (2005) that a low level of heterozygosity is expected where diploid organisms are primarily inbreeds, and can also reproduce asexually (which is the case of *A. zebrina*).

The lack of pattern in clustering and the closeness between populations observed in the present experiment may also have been influenced by the way data were optimized during the current study (see Appendix IV). Proper optimization of samples during selective amplification process is a very important factor to consider, because a peak may exist in a particular sample but may not be evident if it failed to be amplified. The dataset of the present experiment is the best available dataset that could be assembled from available resources. It is, however, recommended that future studies should include further optimization of AFLP molecular markers using adequate repeats of samples.
Several limitations of AFLP have been documented which may have contributed to the lack of pattern observed in the present research. For example, Bensch and Åkesson (2005) have claimed that mutations may result in DNA fragments of different lengths which may cause bands at different positions in the gel, so that two alleles at the same

AFLP locus will be mistakenly scored as the presence of alleles at two different AFLP loci. Secondly, a substitution that creates a new cut site between the primers for any of the two restriction sites may result in the absent allele of one AFLP locus being scored as the presence of an allele at another AFLP locus. The third reported limitation which is a concern particularly in genetic diversity studies is the problem of bands of the same length that are not homologous, and thus represent two or more different AFLP loci. Finally, Weising *et al.* (2005) added that although AFLP is a powerful molecular marker technique approach, it has limitations resulting from the dominance and clustering of markers, particularly in cases with high degrees of polymorphism, which prevents the use of these markers across species boundary.

CHAPTER 7

GENERAL DISCUSSION, CONCLUSIONS AND FUTURE DIRECTIONS

In this chapter, findings of the present study presented in four preceding chapters are re-examined here in general context. This chapter is presented in three sections subdivided into subsections as appropriate. In the first section, general study discussion is presented, which is subdivided into indigenous knowledge on the species existence, utilization, management and propagation, ecological conditions, population status of the species, regeneration and associated plant species, nutritional composition of *A. zebrina* cooked and dried flowers as well as genetic diversity in *A. zebrina* populations. General conclusion of the current study was presented in the second section, while future direction in the third section concluded the chapter.

7.1 GENERAL DISCUSSION

7.1.1 Indigenous knowledge on species existence, utilization, management and propagation

The present study has revealed a significant indigenous knowledge that participants have on the awareness of species existence and status. Variations obtained between respondents for Otjozondjupa and Omusati regions in comparison to respondents in Ohangwena region with regards to the varietal and hermaphroditic of the species indicated the need for educating communities of Ohangwena regions about varietal and hermaphroditic conditions of *A. zebrina*. Indigenous knowledge obtained in Otjozondjupa and Omusati region is similar to what Reynolds (2004) documented. A decrease in species abundance experienced by respondents in Omusati and Otjozondjupa regions during (1987-2007) is in agreement with the study conducted by Somnasang *et al.* (1998) north-east Thailand where villagers experienced a decrease in the availability of wild foods as a result of massive deforestation experienced for years. Although, the issue of deforestation may not be very apparent in Otjozondjupa, it is a fact that deforestation remains one of major environmental problems in Namibia Omusati and Ohangwena regions are included (Harri, 1996) Moreover, a significant increase in the

species abundance reported in Ohangwena region, is in line with findings (Chapter 4 of this thesis), where the highest number of *A. zebrina* plants with highest number of regeneration from seeds was obtained in the same region. This is an addition to the possibility of overexploitation of the species in Otjozondjupa and Omusati regions, which may contribute to declining of the species in these two regions.

Harvesting and utilization of *A. zebrina* flowers confirmed in all the three study regions with (>80%) respondents involved in harvesting the product, therefore reflected high level of utilisation of the species in Namibia; which is in agreement with findings reported by Suich (2003) on the utilization of wild edible plants, where a greater number were consumed in Caprivi region of Namibia. However, discovery of the issue of harvesting permit being only needed in Otjozondjupa and not in other regions were indicating the danger of possible overharvesting of the resource. Therefore, the researcher is of the opinion that *A. zebrina* flowers harvesting permit need to be initiated. The current study has also documented different harvesting times of *A. zebrina* where in Otjozondjupa (May – September), Ohangwena and Omusati regions (January – April). Since the species can be harvested at two different times of the year, there is therefore a potential in propagation and domestication of *A. zebrina* plants in the sense that this would create better opportunity to fully harvest flower products twice a year. However, further research need to be made to establish whether domestication of *A. zebrina* plants in the following ways would work: In the first place if for instance *A. zebrina* plants are to be collected from Otjozondjupa region and be grown in Ohangwena and Omusati regions, and see if these plants would still flower during May to September. Secondly, *A. zebrina* plants are collected from Ohangwena and Omusati and be grown in Otjozondjupa to see if they would flower during January to April.

The main reasons why *A. zebrina* flowers are being harvested for has been unveiled during the current study. The flower products are mostly harvested for home consumption with a provision for sale for harvesters who could obtain surplus, notably women who flock to Otavi area in Otjozondjupa region when the species is flowering in order to harvest flowers as documented by UNAM Research Agenda (2003). These findings are similar to the report by Mulonga (2003) which was conducted in Caprivi region of Namibia that the majority of respondents do harvest wild food resources

mainly for household consumption, while some claimed harvesting them for food and sale with only a single individual harvested them for sale. The study discovery of differences in priorities of harvesting flowers is a very interesting finding. It is an indication that participants in Otjozondjupa region are more in business (generating income from selling flower products) comparing to the case of other regions. Therefore, there is a need to improve the supply of the flower products in Ohangwena and Omusati region, so that people in these communities would have enough flower products for consumption and sale. The popularity of flower cake and *oshinyanekela* in Otjozondjupa and Omusati regions were also unveiled during this research. However, it was interesting to discover that *oshinyanekela* is not known in Ohangwena region, therefore there is a need to introduce this product in Ohangwena region. This is mostly important especially that the cake and *oshinyanekela* are favoured for their natural sweet taste without addition of any other ingredient (>90% respondents) and therefore eaten as a delicacy dish in Namibia and in Kunene Provence of Angola (Larsen, 2000 and Reynolds, 1966).

Another interesting findings discovered during this study is that for one to make a flower cake; flowers have to be collected from several *A. zebrina* plants but not only from a single plant means flowers collected from a single plant would not be enough to make one full cake. However, the slight difference obtained between regions on how much flowers could be collected from a single plant could be influenced by the size of cakes made.

Since *A. zebrina* flower products are eaten as a delicacy; it is not surprising that current research discovered that the majority of respondents in all the three regions purchase flower products for consumption. The highest amount of cakes and spinach purchasing per household was obtained in Otjozondjupa region, this is not surprising because that is where the highest concentration of *A. zebrina* plants are found. Due to the popularity of flower products in this region; it is very common to acquire the product from this region and be sold in other parts of Namibia. The study also discovered that the flower products were only traded locally and the amount traded varied considerably between regions, with the highest amount sold (both cakes and dried 'spinach') per household per harvesting season recorded in Otjozondjupa and the least in Ohangwena. It was also interesting to note that the cost of flower cake and dried 'spinach' was the highest in

Otjozondjupa region, despite its availability in this region as explained by Say's Law which states that supply creates its own demand (Daly, 2004). Since the majority of respondents in Otjozondjupa were selling flower products for income generation, it is therefore important to improve supply of flower products to other regions so that communities of Ohangwena and Omusati regions could also generate income from the sale of *A. zebrina* flower products .

It was also interesting to unveil that goats have topped the list of animals which feeds on *A. zebrina* plants compared to other organisms. This information is important in such a way that propagation and domestication of *A. zebrina* could contribute to animal food supplements especially if chemical components of the species are to be established, which is vital for livestock feed as well as for medicinal value. Although the study discovered that there was limited awareness on the importance of *A. zebrina* plant for medicinal value in all study regions; it was however pointed out that roots were the most important part of the plant considered for medicinal value, followed by the leaves. These findings have therefore necessitated the need for establishing chemical make up of roots and leaves of *A. zebrina*. Claims gathered during this study that conditions treated using *A. zebrina* included expelling the placenta during labour, which was similar to the report on the use of other *Aloe* spp. in postnatal care documented by Grace *et al.* (2008) in Southern Africa. It is therefore suspected that there might be something that causes contractions of the uterus (Dr. E. Kamati, personal communication, 27 November 2009). It is also wise to know the kinds of animals one should keep off *A. zebrina* plants during growing, tending, harvesting and processing of flower products.

The study has also find that the majority of flowers in Ohangwena and Omusati regions are collected from communal land while in Otjozondjupa regions; flower harvesting is mostly from privately owned commercial farms in the vicinity of Otavi area. This means propagation and domestication of *A. zebrina* plants in Ohangwena and Omusati regions may give local communities in these areas a better access to harvesting flowers, especially if the species is to be grown in communal area and also on private land. Moreover, communities in Otjozondjupa region could also be encouraged to grow the species on their own land since currently; they only harvest flowers from private commercial farmers' land. Domestication of *A. zebrina* plants would be important to

both men and women especially in Otjozondjupa and Omusati region where both men and women played important role in harvesting and selling of flower products. However, in Ohangwena region the majority of responded have reported that harvesting of *A. zebrina* flowers is a women's role. This is probably the reason why local women vendors were found to be the major suppliers of *A. zebrina* flower products in all three study regions. Which is in line with the report by (Harris and Salisu, 2003) who documented that women have more knowledge on wild foods than men, which could be the reason why they are primarily responsible for collection and preparation of wild foods in many rural communities? Since the study unveiled that *A. zebrina* can be easily domesticated from seeds, which may offer an alternative to uprooting of wildlings and arrest further erosion of the germplasm of this important resource in Namibia; this means women are the main stakeholders in propagation and domestication of *A. zebrina* plants.

7.1.2 Ecological conditions, population status, regeneration and *A. zebrina* associated plant species

The study has revealed the ecological conditions of mean annual rainfall ranged between 370 and 472 mm and the mean annual temperature ranged between 20.4 and 23.1°C, both of which are characteristic of dry land ecosystems reported to be the ecological requirements for *A. zebrina*. However, Faucon (2005) has reported that the species prefers minimum average temperature of about 10°C, while SEPASAL (2007) and Backyardgardener (2008) reported that a semi-arid climate is the species' major ecological requirement with subtropical, hot and arid climate also documented (SEPASAL, 2007). Moreover, the researcher still believes that the species can grow under various ecological conditions especially considering its distribution range throughout Southern Africa (Figure 2.3).

The study also finds that sandy soil is the dominant soil type in all the study regions, with Ohangwena region showed significantly the highest sand content with the highest number of plants per hectare and largest sized plants in terms of height and diameter. However, the fact that the majority of sampling plots in Ohangwena and Omusati were based on semi-domesticated plants; this may have contributed to the highest number of plants per hectare and largest sized plants in terms of height and diameter recorded in

Ohangwena region. This is in line with what Erickson *et al.* (2005) documented that plants adapt to morphological changes when domesticated and these changes usually distinguish them from their wild relatives. The strong and positive correlation obtained between plant parameters and sand content of soil is an indication that sandy soil is the most suitable soil condition for *A. zebrina* as confirmed by Rothmann (2004), SEPASAL (2007) and Backyardgardener (2008). This means *A. zebrina* prefer the soil type consist of large gritty soil particles, which enable water to drain through and dry out quickly, where soil air is plentiful but the particles themselves lack nutrient value (Garden Barn, 2009). These are interesting results especially that *A. zebrina* plants are mostly concentrated in Otjozondjupa where the highest clay and silt soil particles were found as well as highest nitrogen and organic matter content were recorded. Since soil characteristics for Otjozondjupa were slightly different from that of Ohangwena, simply because in Otjozondjupa the soil particles are fine and sticky; drainage and soil air content are poor and the soil particles contain plenty of nutrients (Garden Barn, 2009) comparing to sandy soil. There is a need to further investigate about the factors contributing to generally high concentration of the species in Otavi area as document by UNAM Research Agenda (2003).

Omusati had the highest contents of potassium, calcium, magnesium and sodium (mg kg^{-1}), and the highest soil electrical conductivity 607.4 $\mu S\ cm^{-1}$) therefore means high cation holding capacity of the soil as stated by (Busman, 2002). Since Cation Exchange Capacity (CEC) is basically defined as a rating of the soil's ability to hold nutrients or a fertility-holding capacity (BioFlora, 2009), and soils with high CEC are usually significant in clay or organic matter content; that are likely to have poor internal drainage as well as soil compaction in high traffic areas (Turf Revolution, 2009). Therefore, suggesting that soils in Omusati are not very best suitable for growing *A. zebrina*.

The study has also discovered that the highest number of *A. zebrina* regeneration per hectare was recorded in Omusati with the highest number of plants regeneration from roots was recorded in Otjozondjupa while the most regeneration from seeds was recorded from Ohangwena region. This is suggesting that the current harvesting practice used especially in Otjozondjupa is unsustainable and may threaten the species with extinction. Therefore, better sustainable harvesting practices must be initiated in order to

save the species from extinction. *A. zebrina* associated plant species (trees and shrubs) dissimilarities showed during the current study may reflect variations between the three study regions, which did not have impact on the species existence.

7.1.3 Nutritional composition of *Aloe zebrina* cooked and dried flowers

This study has also established the nutritional content of *A. zebrina* flower product. Nutritional components obtained are such as fat (1.5), protein (14.22), dietary fibre (54.5), magnesium (26), calcium (6), potassium (86.1), sodium (11.4), zinc (0.6 g), manganese (0.2), iron (3.2) and phosphorus (0.1) g per 100 g of *A. zebrina* flower product. The fat content of *A. zebrina* flower product per 100 g of the product is well within the range of composition values reported for most food items (Appendix VIIII) and is comparable to the fat content of 100 g for boiled brown rice of 1.4 g and that of white bread of 1.6 g. This indicates that *A. zebrina* flower products are rich in protein, dietary fibre, magnesium with the values of calcium, potassium and sodium obtained were also higher compared to other vegetables such as cabbage (0.7 Ca: 2.7 K: 0.1 Na) g per 100 g; lettuce (0.7 Ca: 4.2 K: 0.1 Na) g per 100 g and spinach (1.4 Ca: 7.4 K: 0.6 Na) g per 100 g, respectively.

The study has unveiled that 100 g of *A. zebrina* flower product could provide the daily zinc requirement for 56 people. This value was also similar to value of 0.5 g reported by Bear *et al.* (1948) for raw stalks of Asparagus and boiled leaves of beetroot. Although, the content of Manganese from 100 g of *A. zebrina* flower product could provide the daily requirement for 15 people, its value was however, lower than that of tomatoes (4.5 g) as documented by Bear *et al.* (1948). The content of iron obtained from 100 g of *A. zebrina* flower product was high comparing to the content of iron in cooked cabbage (0.3) and lettuce (0.5) mg per 100 g as reported by Gebhardt (2002), while the content of phosphorus obtained from 100 g of *A. zebrina* flower product can only provide 3% of P required per day (Table 5.4). Although the amount of ash (5.3%) found in 100 g of *A. zebrina* flower product was within the range reported in literature, it was however low comparing to findings of the study conducted by Bear *et al.* (1948) where ash content in cabbage was 8.2%, lettuce 11.5% and spinach 26.1% was recorded.

The B complex vitamins such as nicotinic acid (5.4), pyridoxine (2.6) and thiamine (1.0) were discovered (numbers in brackets are mg per 100 g of *A. zebrina* flower product). *A. zebrina* flower have a large amount of these vitamins compared to the values reported by Gebhardt (2002) of thiamine and nicotinic vitamins for cooked cabbage and lettuce (0.09 mg thiamine and 0.4 mg nicotinic acid) and (0.10 mg thiamine and 0.5 mg nicotinic acid), respectively.

7.1.4 Genetic diversity in *Aloe zebrina* populations

The results of a genetic diversity study based on the total number of bands (359) produced from eight combined primer pairs indicated an overall Polymorphism of 86% with an average of 39 loci per primer combinations. This suggests a high degree of diversity between individual *A. zebrina* plants in Namibia. A dendrogram, however, showed no significant grouping of *A. zebrina* populations, neither by study sites nor by study regions. This means that individual samples collected from all three study regions were found spreading all over constructed dendrogram and no pronounced grouping was observed (Figure 6.2).

Since *A. zebrina* is found growing in clusters and reproduce by rhizomes, this may increase the possibility of inbreeding which is likely to decrease overall diversity. The mean pairwise Jaccard's distance coefficient between *A. zebrina* populations in the three study regions showed 0.27 (0.0 = completely different) and 0.73, (1.0 = homozygous). This is in agreement with what was reported by Kosman and Leonard (2005) that a low level of heterozygosity is expected where diploid organisms are primarily inbreeds, and can also reproduce asexually (which is the case of *A. zebrina*).

The lack of pattern in clustering and the closeness between populations observed in the present experiment may also have been influenced by the way data were optimized during the current study (see Appendix VIIII). Proper optimization of samples during selective amplification process is a very important factor to consider, because a peak may exist in a particular sample but may not be evident if it failed to be amplified. The dataset of the present experiment is the best available dataset that could be assembled

from available resources. It is, however, recommended that future studies should include further optimization of AFLP molecular markers using adequate repeats of samples.
Several limitations of AFLP have been documented which may have contributed to the lack of pattern observed in the present research. For example, Bensch and Åkesson (2005) have claimed that mutations may result in DNA fragments of different lengths which may cause bands at different positions in the gel, so that two alleles at the same AFLP locus will be mistakenly scored as the presence of alleles at two different AFLP loci. Secondly, a substitution that creates a new cut site between the primers for any of the two restriction sites may result in the absent allele of one AFLP locus being scored as the a presence of an allele at another AFLP locus. The third reported limitation which is a concern particularly in genetic diversity studies is the problem of bands of the same length that are not homologous, and thus represent two or more different AFLP loci. Finally, Weising *et al.* (2005) added that although AFLP is a powerful molecular marker technique approach, it has limitations resulting from the dominance and clustering of markers, particularly in cases with high degrees of polymorphism, which prevents the use of these markers across species boundary.

7.2 CONCLUSIONS

7.2.1 Indigenous knowledge, utilization, management and propagation of *Aloe zebrina*

- From socio-economic survey of assessing *A. zebrina* resource, its utilization and management; it can be concluded that, participants in Otjozondjupa, Ohangwena and Omusati regions are knowledgeable of *A. zebrina* species ranging from the existence of the species and its status, its importance as food, medicine as well as animal feed. Vast knowledge gathered during present study included processing of flower products, production and consumption, taste, demand, supply and cost; markets and income generation, *A. zebrina* plant as animal food resource, medicinal importance, nutritional importance, gender role, land tenure with regards to ownership as well as location where the flowers are collected, domestication, propagation and management of *A. zebrina* species.

- Results on the observations made on testing propagation of *A. zebrina* species from seeds showed the potential in growing the species using tested low-cost propagation methods which were demonstrated during the present study.

7.2.2 Ecology, population status, regeneration and associated plant species

- Based on the climatic data gathered during current study, sandy soil is the most preferable soil for growing *A. zebrina* species. The study has unveiled that too much silt, clay; N as well as OM in the soil will affect the growth and development of *A. zebrina* plants. Regeneration of species is affected by over collection of flowers; which does not give a chance to seed production, therefore make the species being threatened. Conservation strategies for intervention need to be put in place before the species disappear completely from the Namibian communities notably from Ohangwena and Omusati region. Similarities in plants associated to *A. zebrina* species observed in the study area, indicates the suitability of these species in the area under study.

7.2.3 Nutritional composition of *Aloe zebrina* cooked and dried flowers

- From the nutritional composition study of *A. zebrina* flower products, it can be concluded that the species is nutritional rich, which can cater for most of nutritional needs. Therefore, conservation, domestication and propagation of *A. zebrina* species would with no doubt improve both nutrition and health of the Namibian nation, for communities in Kunene Provence in Angola as well as for livestock farming.

7.2.4 Genetic diversity in *Aloe zebrina* populations

- Further research need to be conducted in order to determine the status of genetic diversity between and within *A. zebrina* populations.

7.3 FUTURE DIRECTIONS

Based on the present study the following actions are to be implemented by the government of the Republic of Namibia, scientists and researchers, flower collectors, relevant institutions, NGOs, educational trainers and extension officers.

First, since the study has established that, participants in Otjozondjupa, Ohangwena and Omusati regions are knowledgeable of *A. zebrina* species ranging from the existence of the species and its status, its importance as food, medicine as well as animal feed. This includes the potential of growing the species using tested low-cost propagation methods demonstrated during the present study. Therefore, there is a need to initiate propagation as well as domestication of the species, so that local people would get more benefit from this species. This can be achieved through establishment of *in-situ* and *ex-situ* conservation strategies by scientists of the University of Namibia on propagation and domestication of *A. zebrina* species in Namibia. The wide range of indigenous knowledge gathered on processing of flower products, production and consumption, taste, demand, supply and cost, markets and income generation, nutritional importance, gender role, land tenure and location where the flowers are collected; these are multi-informative information where several activities based on the importance of *A. zebrina* species could be drawn such as adding value to the flower products, establishment of markets for trading the flower products and generation of income from selling plant products. This has to be implemented by flower collectors supported by the government.

There is also a need to establish chemical components of *A. zebrina* species, leaves as well as roots in order to further research the species potential in medicinal and nutritional use. This can be achieved by scientists of the University of Namibia in collaboration with relevant institutions at national and international level.

Secondly, although the present study has established that local people are somehow managing the species, there is a need for communities to change the way they propagate *A. zebrina* plants that is by general wildlings any *A. zebrina*, especially in Ohangwena and Omusati regions. This can be achieved through educating communities by conducting seminars, workshops, and the use of mass media as supported by Mulonga

(2003) that teaching communities about the environment and sustainable harvesting of resources is important for increased awareness of sustainable utilisation. This can be spearheaded by training of trainers in association with extension office services as well as relevant NGOs.

Thirdly, the similarity in ecological conditions in the study area is the best indication of the species suitability to the area. However, slightly difference in population status, regeneration as well as plant morphological characteristics need further investigation. This can be achieved by scientists conducting further research investigating into the possible causes of these variations.

Fourthly, although the present study has established the availability of proteins, dietary fibre, ash, vitamins (B complex), fat and minerals such as calcium, potassium, magnesium, phosphorus, sodium, iron, magnesium and zinc in cooked and dried *A. zebrina* flowers; it is recommended that further research based on nutritional value of flower products need to be done in order to bring more nutritional components onboard such as determination of energy, carbohydrates (specifying sugars content), fat (including establishment of how much are saturate), other vitamins such as Vitamin A and C. This can be done by Namibian researchers in collaboration with relevant institutions at national and international level as well as NGOs.

Fifth, since there is limited information based on *Aloe* plants molecular studies, although the present study has at least established baseline information on the genetic diversity study between *A. zebrina* populations and further investigation into this subject was recommended; a broad study comprised of genetic diversity between *A. zebrina* and other *Aloe* spp., which are similar to *A. zebrina* such as *A. esculenta*, *A. saponaria* (*maculata*), *A. littoralis*, *A. greatheadii*, *A. parvibracteata* and *A. angolensis* (which is a hybrid), fingerprinting and sequencing need to be done.

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APPENDICES

Appendix I Questionnaire (Individual) qualitative methods

Socio-economic survey and feasibility study of *Aloe zebrina* Baker to measure its potential for domestication, nutritional content and possibility of income generation in Namibia.

Region_____

Study site_____ **Date**_____

Respondent name_____ **Gender**_____

Name of enumerator_____

A. Species existence and population status

1. Does *Aloe zebrina* Baker (*ekundu*) species occur in this area?

Yes (....)

No (....)

No idea (....)

2. Do you know if the species has more than one variety?

Yes (....)

No (....)

No idea (....)

If yes, describe them.

_____

_____

_____

_____

____

3. Do you uproot the species?

Yes (....)

No (....)

If yes, for what purpose? _____

4. Did you notice any change in the species abundance in the past 20 years?

_____

_____

—

B. Importance of *Aloe zebrina* Baker

5. Do you pick *Aloe* flowers?

Yes (....)

No (....)

6. Do you require any harvesting permit to pick *Aloe* flowers?

Yes (....)

No (....)

7. How much (number of cakes) is collected from a single plant?_____

8. What time of the year does harvesting of flower carried out? _____

9. Why do you harvest aloe species?

Home consumption (....)

For sale (....)

B (i) Household consumption

10. How much processed aloe flowers consumed per household per year?

Cakes (No. of cakes) _____

Dried spinach (kg) _____

11. Explain the procedure followed to preserve *Aloe* flowers, starting from picking:

Cakes

_____

_____

_____

_____

_____

_____

_____

_____

_____

Dry 'spinach'

_____

_____

_____

_____

_____

_____

_____

_____

12. When harvesting aloe flowers, do you selection from which plant to pick flowers?

Yes (....)

No (....)

No idea (....)

If yes, give the reason why _____.

B (ii) Marketing

13. Do you buy processed *Aloe* flowers?

Yes (....)

No (....)

If yes, where do you buy them?

_____

14. How many cakes do you buy per season for your household? _____

15. How much 'dried spinach' do you buy per season? _____(kg)

16. Does marketing of processed aloe flowers readily available?

Yes (....)

No (....)

No idea (....)

If no, how can it be improved? _____

17. How much does processed aloe flowers cost?

Price / cake _____

Price / dried flowers _____ (specify tin size)

B (iii) Income generation

18. Where do you sell processed aloe flowers? _____

19. How many cakes do you sell per season?_____

20. How much 'dried spinach' do you sell per season? _____(kg)

21. Does marketing of processed aloe flowers readily available?

Yes (....)

No (....)

No idea (....)

If no, how can it be improved? _____

22. How much does processed aloe flowers cost?

Cost/ cake _____

Cost/ dried flowers _____ (specify tin size)

B (iv) Livestock

23. Does *Aloe zebrina* eaten by Livestock?

Yes (....)

No (....)

No idea (....)

24. Which part of the plant eaten by which livestock class?

Animal	Plant part
Cattle	_____
Goat	_____
Sheep	_____
Donkey	_____
Horse	_____

Chicken _____

Other _____

C. Gender role

25. Who does the harvesting?

Men (....)

Women (....)

Boys (....)

Girls (....)

No idea (....)

D. Land tenure

26. Where do you pick *aloe* flowers?

In the field (cultivated land) (....)

In the field (uncultivated land) (....)

Outside the field (outside field fence) (....)

Other (how far in km)? (....) _____

27. Who own that land?

E. Medicinal value analysis

28. Does *Aloe zebrina* have any medicinal importance?

Yes (....)

No (....)

No idea (....)

29. Which part of the plant used?

_____

_____

_____

_____

____

F. Domestication, propagation and management aspect of the species

30. Do you think that *A. zebrina* can be domesticated?

Yes (....)

No (....)

No idea (....)

Explain_____

_____

_

31. Have you ever tried to propagate this species?

Yes (....)

No (....)

32. What methods could be used in planting the species?

a) Seeds

Explain_____

_____

_____

_____

___

b) by cuttings

Explain_____

_____

_____

_____

___

c) other

Explain_____

_____

_____

___

33. What kind of management aspects you practice on the species?

34. Age category (yrs)

0-30

31-45

46-60

61-75

76-90

91+

Appendix II Questionnaire (group interviews) quantitative methods

Socio-economic survey and feasibility study of *Aloe zebrina* Baker to measure its potential for domestication, nutritional content and possibility of income generation in Namibia.

Region_____

Study site_____ **Date**_____

Gender_____

Farmers will be interviewed in groups, whereby informal discussion will be carried out in smaller groups (3 groups per site). Participants will be discuss the following questions and present their results. The Researcher and her assistant will facilitate the discussion.

1. Did you notice any change in the species abundance in the past 20 years?
2. Do people require any harvesting permit to pick *Aloe* flowers?
3. What time of the year does harvesting of flower carried out?
4. Explain the procedure followed in preserving *Aloe* flowers into cakes and dry 'spinach', starting from picking process?
5. Does marketing of processed aloe flowers readily available? If not, how can it be improved?
6. Does *Aloe zebrina* eaten by Livestock and which part of the plant eaten by which livestock class?
7. What is the cost of processed aloe flowers, (price/cake and dried flowers)?
8. What is the importance of *A. zebrina* from nutritional point of view?
9. Does *Aloe zebrina* have any medicinal importance? Which part of the plant used?
10. Explain why you think that *A. zebrina* can be domesticated and what methods could be used to plant the species?
11. State any kinds of management aspects that can be practiced on the species?
12. Is there any risk involved during the harvesting?

13. Have you ever heard / experienced any case whereby somebody was irritated by eating 'ekundu'?
14. Do you have any comment on the importance of 'ekundu'?

Appendix III Food composition table for fat and protein content of some foods.

Food (100g)	Protein(g)	Fat(g)
All Bran	13	2.5
Almonds	17	54
Alpen	11.5	6.2
Apples	0.2	0
Apricots	0.6	0
Apricots, dried, raw	4	0
Artichokes Globe boiled	1	0
Artichokes Jerusalem boiled	1.5	0
Asparagus boiled	1.7	0
Aubergine raw	0.7	0
Avocados	4	20
Bacon collar joint boiled	20	27
Bacon gammon grilled	30	12
Bacon gammon joint boiled	25	19
Bacon rashers streaky fried	23	45
Bacon rashers streaky grilled	25	36
Baked Beans - Heinz	5	0.3
Baked beans - Weight Watchers	4.8	0.3
Bananas	1	0.3
Barcelona nuts	11	64
Barley pearl boiled	2.7	0.5
Bean sprouts canned	1.6	0
Beans broad boiled	4	0.6
Beans butter boiled	7	0.3
Beans french boiled	0.8	0
Beans haricot boiled	6.6	0.5
Beans mung cooked	6	4
Beans runner boiled	2	0.2
Beef brisket boiled	27	24
Beef Casserole -Crockpot	9.87	3.37
Beef corned canned	26	12
Beef minced stewed	23	15
Beef rump steak fried	29	15
Beef rump steak grilled	27	12

Beef silverside salted	29	14
Beef sirloin roast	24	21
Beef steak stewing stewed	30	11
Beef topside roast	27	12
Beer bitter	0	0
Beer larger	0	0
Beer stout	0	0
Beetroot boiled	1.8	0
Beetroot raw	1.3	0
Bilberries	0.5	0
Biscuit - Custard Cream	6	23.8
Biscuit - mini cheddars	9.8	29.1
Biscuits - morning coffee	6.9	14.5
Biscuits - Rich Tea	6.9	15.7
Biscuits Chocolate covered	6	27
Biscuits digestive chocolate	7	24
Biscuits digestive plain	6.5	22
Biscuits semi sweet	7	17
Biscuits shortbread	6	26
Biscuits wafers filled	5	30
Biscuits water	11	12.5
Black Currants	1	0
Black pudding fried	13	22
Blackberries	1.5	0
Bran Flakes	9.3	2
Bran wheat	14	5.5
Brazil Nuts	12	60
Bread brown	9	2.2
Bread malt	8	3
Bread rolls white	10	7
Bread white	7.8	1.6
Bread white toasted	9.6	1.7
Bread wholemeal	8.8	2.7
Breadcrumbs white	12	2
Broccoli tops boiled	3	0
Brussels sprouts boiled	3	0
Buns current	7	8
Butter	0.5	82

Cabbage savoy boiled	1.3	0
Cabbage savoy raw	3	0
Cabbage spring boiled	1	0
Cabbage winter boiled	1.7	0
Cake fruit	4	11
Cake Madeira	5	17
Cake rock	5	16
Cake sponge with fat	6.5	27
Cake sponge without fat	10	7
Cakes fancy	4	15
Carrots boiled	0.6	0
Carrots raw	0.7	0
Cauliflower boiled	1.5	0
Cauliflower cheese	6	8
Celery boiled	0.6	0
Celery raw	1	0
Cheese camembert	23	23
Cheese cheddar	26	34
Cheese cottage	14	0.5
Cheese cream	3	47
Cheese danish blue	23	29
Cheese edam	24	23
Cheese parmesan	35	30
Cheese processed	22	25
Cherries	0.5	0
Cherries glace	0	0
Cheese stilton	26	40
Chestnuts	2	2.7
Chicken Kiev	24.4	21.6
Chicken roast boned	25	5
Chicken roast meat	22	7.5
Chicory	0.8	0
Chocolate Break (mug of)	3.1	2.4
Chocolate milk	8	30
Chocolate plain	5	29
Christmas Pudding	5	12
Cider dry	0	0
Cider sweet	0	0

Cockles boiled	11	0.3
Cocoa powder	19	22
Coconut	3	36
Coconut desiccated	6	62
Cod fillet baked	21	1.2
Cod fillet fried	21	8
Cod fillet fried in batter	20	10
Cod fillet grilled	21	1.3
Cod fillet poached	21	1
Cod fillet steamed	19	1
Coke diet	0	0
Cola	0	0
Coleslaw	1.1	14.8
Cornflakes	8	0.5
Cornflour	0.6	0.7
Cornish pastie	8	20
Crab boiled	20	5
Crab canned	18	1
Cracker Bread	10.9	4.1
Cranberries	0.5	0
Cream crackers - Crawford	7.7	12.8
Cream double	1.5	48
Cream single	2.4	21.2
Cream sterilised canned	2.6	23
Cream whipping	1.9	35
Crispbread rye	9.5	2
Crispbread wheat	45	7.5
Croissant	4.3	12.2
Cucumber	0.6	0
Currants dried	2	0
Custard egg	6	6
Custard powder	4	4
Damsons	0.4	0
Dates dried	2	0
Drinking chocolate	6	6
Dripping	0	100
Duck roast	25	10
Dumpling	3	12

Egg dried whole	43.6	43.3
Egg scrambled	10	23
Egg whole raw	12.3	10.9
Egg yolk	16.1	30.5
Eggwhite	9	0
Fat cooking	0	100
Fish fingers fried	13.5	13
Flour brown	13	2
Flour white	11	1.2
Flour white self raising	9	1.2
Flour wholemeal	13	2
Fruit gums	1	0
Fruit juice sweetened	0	0
Fruit juice unsweetened	0	0
Fruit pie	4	16
Fruit salad canned	0.3	0
Goose roast meat	29	22
Gooseberries	1	0
Grape nuts cereal	11	3
Grapefruit - Tin	0.3	0
Grapefruit peeled	0.5	0
Grapes black	0.5	0
Grapes white	0.6	0
Green Bean Mix	1.21	0.53
Greengages	1	0
Haggis boiled	11	22
Halibut steamed	24	4
Ham	18	5
Ham cooked	24.7	18.9
Hamburgers fried	20	17
Hare stewed with bones	22	6
Heart roast	26	15
Heart stewed	31	6
Herring grilled	20	13
Honey	0	0
Ice cream dairy	4	7
Ice cream non dairy	3	8
Jams	0.5	0

Jelly packet	1	0
Kidney fried	25	6
Kidney stewed	26	8
Kipper baked	26	11
Lamb breast roast	19	37
Lamb chops loin grilled	24	29
Lamb cutlets grilled	23	31
Lamb leg roast	26	18
Lamb scrag and neck stewed	26	21
Lamb shoulder roast	20	26
Lard	0	100
Leeks boiled	1.8	0
Lemon curd	0	5
Lemon juice	0.3	0
Lemon sole fried in breadcrumb	16	13
Lemon sole steamed	21	1
Lemonade bottled	0	0
Lemons	1	0
Lentils boiled	8	0.5
Lettuce	1	0
Liver fried	27	13
Liver stewed	25	10
Lobster boiled	22	3.5
Luncheon meat	13	27
Macaroni boiled	4	0.6
Macaroni cheese - Tin	3.6	4.8
Mackerel fried	22	11
Mandarin Oranges - can	0.6	0
Mango Chutney	0.5	0.4
Mangoes	0.5	0
Macaroni cheese	7	10
Margarine	0	81
Margarine low fat spread	0	40
Marmalade	0	0
Marrow boiled	0.4	0
Mars bar	5	19
Marzipan	9	25
Mayonnaise	2	79

Melon	0.5	0
Milk	3.3	3.8
Milk condensed skimmed	9.9	0.3
Milk condensed whole sweetened	8.3	9
Milk evaporated whole	8.6	9
Milk skimmed	3.4	0.1
Mince and Spaghetti	8.79	4.04
Mincemeat	1.0	4
Muesli	13	7.5
Mushrooms fried	2.2	22
Mushrooms raw	2	0.6
Mustard and cress	1.5	0
Nectarines	1	0
Oil vegetable	0	100
Omelet	11	16
Onions fried	2	33
Onions raw	1	0
Onions spring	1	0
Orange juice	0.6	0
Orange peeled	1	0
Oxo cube	1.5	0.3
Pancakes	6	16
Parsnips - boiled	1.3	0
Pasta	13.2	2
Pastry flaky cooked	6	40
Pastry Puff	5.4	25
Pastry shortcrust cooked	7	32
Peach Slices - tin	0.5	0
Peaches canned	0.5	0
Peaches fresh	0.5	0
Peanuts fresh	24	50
Peanuts roasted and salted	24	50
Pears	0.2	0
Peas boiled	5.4	0.4
Peas canned	5	0.3
Peppers green raw	1	0.4
Philadelphia cheese - light	12	15
Pickle Branston	0.7	0.2

Pilchards canned	19	5
Pineapple canned	0.4	0.2
Pineapple fresh	0.5	0
Plaice fried in butter	16	18
Plaice steamed	19	2
Plums cooking	0.5	0
Plums dessert	0.5	0
Popcorn	1.6	12.8
Pork chops grilled	29	24
Pork leg roast	27	20
Porridge Oats with Bran	10.6	6.7
Potato chips	4	11
Potato crisps	7.3	35.2
Potatoes baked with skin	2	0
Potatoes boiled - King Edwards	1.4	0.1
Prawns	23	2
Prunes - Tin	1	0
Prunes stewed	1	0
Puffed wheat cereal	14	1.3
Quiche Lorraine	15	28
Rabbit stewed	14	4
Radish	1	0
Raisins	1	0
Raspberries	1	0
Red Currants	1	0
Rhubarb raw	0.5	0
Rhubarb stewed with sugar	0.5	0
Rice Brown Boiled	3.4	1.4
Rice Krispies cereal	6	0.7
Rice pudding canned	3.7	1.2
Rice white boiled	2.6	0.1
Rice white raw	6.5	1
Salad Cream	2	27
Salad Cream Weight Watchers	1.2	8.2
Salmon steamed	20	13
Sardines canned in oil	24	14
Sardines canned tomato sauce	18	12
Satsumas peeled	1	0

Sauce brown	1	0
Sausages beef grilled	13	17
Sausages pork grilled	13	25
Sausage - Herta	12	31
Scampi fried in breadcrumbs	12	18
Scones	7.5	15
Sherry dry	0	0
Sherry sweet	0	0
Shredded wheat cereal	10.5	3
Sorbet Blackcurrent	0.3	0
Spaghetti boiled	4	0.3
Spaghetti canned tomato sauce	1.7	0.2
Spaghetti tin, weight watchers	1.7	0.2
Spirits - whisky, Gin etc	0	0
Sponge pudding	6	16
Spring greens boiled	1.7	0
Squash undiluted	0.1	0.1
Squash Undiluted - sugar free	0.1	0
Steak stewed canned	15	13
Strawberries fresh	0.5	0
Sugar	0	0
Sugar puffs cereal	6	0.8
Sultanas	2	0
Swede boiled	1	0
Sweetcorn	4.1	2.3
Sweets boiled	0	0
Tangerines peeled	1	0
Toffees	2	17
Tomato juice	0	0
Tomato Ketchup	1	0
Tomato Puree	5.6	0.4
Tomatoes canned	1	0
Tomatoes raw	1	0
Trout steamed	24	4.5
Tuna canned in oil	25	0.1
Turkey roast	29	3
Turnips boiled	0.7	0
Veal fillet roast	32	12

Venison roast	35	6
Walnuts	11	52
Watercress	3	0
Weetabix cereal	11.5	3.5
Wine red	0	0
Wine white dry	0	0
Wine white sweet	0	0
Yogurt flavoured - low fat	4.6	0.1
Yogurt natural	5.9	1.2
Yorkshire puddings	7	10

Source: Brianmac (2009), with modification.

Appendix IV DNeasy protocol: Purification of Total DNA from Plant Tissue (*NB: If a new kit is being used, ETHANOL must be added to AW buffer!!*)

General preparation

- Buffer AP1 and Buffer AP3/E concentrate may form precipitates upon storage. If necessary, warm to 65°C to dissolve (before adding ethanol to Buffer AP3/E). Do not heat Buffer AP3/E after ethanol has been added.
- Buffer AW and Buffer AP3/E are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96-100%) as indicated on the bottle to obtain a working solution.
- Preheat a water bath or heating block to 65°C
- Collect a container of liquid nitrogen
- Collect a container of ice
- Collect plant materials in Petri-dishes
- Collect pestles, mortars, spatula (all sterilized to avoid contamination)
- Collect tube racks, razor blades, measuring cylinder, pipettes, scissor – to cut blue tips
- Collect tips (blue, yellow and clear ones) and a stop watch
- Prepare 70% ethanol for cleaning
- Set up tubes – 1 Lilac QIAshredder, 1 DNeasy spin column, 1 micro-centrifuge tube (1.5 ml), collection tube (2ml) two for each sample .

Sample preparation

Before extracting DNA, *A.zebrina* leaves were prepared as follows:

- Using scalpel blade and a tissue forceps cut off thorns along the leaf margins.
- Then split the leaf into a symmetry (dividing the upper part of the leaf from the bottom part.
- Scrap out a junk of gel and remove as much water as possible, but avoid removing most of leaf inner tissues

- Cut cleaned leaf tissues into dices to facilitate better grinding

Procedure

1. Weigh out 0.1000g fresh plant tissue directly.
2. Put it into a chilled mortar containing liquid nitrogen and grind well with chilled pestle (remember to chill the spatula as well).
3. Transfer ground tissue into 1.5 ml micro-centrifuge tube.
4. Add 400 µl Buffer AP1 and 4µl RNase A stock solution, and vortex to remove crumps. No tissue crumps should be visible – repeat vortex/pipette mix if necessary or use micro-pestle.
5. Incubate the mixture 65 ^{o}C for 10min to lyse cells. Mix by inverting tube 2-3 times during incubation by inverting tube.
6. Add 130 µl Buffer AP2 to the lysate, mix and incubate for 5min on ice.
7. Recommended: Centrifuge the lysate for 5min at 13, 2000 rpm.
8. Pipet all the lysate (with cut blue tip) into lilac QIAshredder Mini spin column placed into 2ml collection tube and centrifuge for 2min at 13,2000 rpm
9. Transfer the flow-through fraction from step 8 into a new (2ml collection tube) without disturbing the cell-debris pellet. (Typically 450 µl of lysate is recovered)
10. Add 1.5 volumes of Buffer AP3/E to the cleared lysate, and mix by pipetting. (For example, for 450 µl lysate, add 675µl Buffer AP3/E. Reduce the amount of Buffer AP3/E accordingly if the volume of lysate is smaller).
11. Pipet 650 µl of the mixture from step 10, including any precipitate that may have formed, into the DNeasy Min spin column placed in a 2ml collection tube and (retain the left over mixture in the collection tube to be used in step 12). Centrifuge for 1min at 8000 rpm for most micro-centrifuges, and discard the flow-through. Reuse the collection tube in step 12.
12. Repeat step 11 with remaining sample. Discard flow-though and collection tube.
13. Place the DNeasy Mini spin column into a new 2ml collection tube and add 500 µl Buffer AW, and centrifuge for 1min at 8000 rpm. Discard the flow-through and reuse the collection tube in step 14.

14. Add 500 µl Buffer AW to the DNeasy Mini spin column, and centrifuge for 2min at 13, 2000 rpm to dry the membrane.
15. Transfer the DNeasy Mini spin column to 1.5 ml micro-centrifuge tube and pipet 100 µl Buffer AE directly into DNeasy membrane. Incubate for 5min at room temperature (15-25), and then centrifuge for 1min at 8000 rpm to elute.
16. Repeat step 15 once.
    i) A new micro-centrifuge tube (1.5 ml) can be used for the second elution step to prevent dilution of the first eluate.
    ii) Alternatively, the micro-centrifuge tube can be reused for the second elution step to combine the eluates.

    In this study, the second option has been employed to get better DNA concentration.
17. Store DNA samples in the freeze.

Appendix V 1% mini agarose gel (after extraction)

(*NB: Use gloves all the time, ethidium bromide are a very toxic chemical!!*)

1. Prepare gel tray and comb.
2. Prepare 1 litre (1000ml) of 1 x TAE electrophoresis buffer (by adding 1 ml of 50 x TAE electrophoresis buffers, which give final volume 20 ml of TAE).
3. Weigh out 1g of agarose and put it into 250ml conical flask containing 100ml of 1 x TAE buffer.
4. Microwave for about 1 min to dissolve the agarose.
5. Cool the gel solution to about 55°C by swirling under running water or leave it to stand on the bench for 5 min down to 50-60°C.
6. Add 2.5µl of ethidium bromide and swirl to mix(final concentration is 0.5µg/ml)
7. Pour the warm agarose gel in a gel tray. Push any bubbles away to the side with a disposable tip. Insert the comb and double check that it is correctly positioned.
8. Leave it on the bench to set for about 20 min.
9. Gently remove the comb from the gel, put the gel tray into the electrophoresis tank and fill it with enough 1 x TAE electrophoresis buffers to submerge the gel for at least 3-5 mm.

Preparing samples

10. Transfer an appropriate amount of each DNA sample (in this case 5 µl) to a fresh micro-cetrifuge tube.
11. Add an appropriate amount of loading dye (1-2 µl) into each tube by mixing well and leave the tip in the tube (to be used in loading sample onto gel tray). That is 5 parts DNA: 1-2 parts loading dye.
12. Load the first well with 5 µl of marker (200bp Ladder).
13. Continue loading samples and take note of physical order of the tubes to be able to identify lanes on the gel photograph.
14. Close the tank, switch on the power-pack and run the gel at 60V for 30-45min.
15. Check that a current is flowing (by looking at the electrodes if evolving gas bubbles).

16. Monitor the progress of the gel by reference to the marker dye.
17. Visualize DNA bands on UVitec gel documentation system.
18. Record your results.
19. Then, DNA samples were stored in a freeze / freezer.

Appendix VI 1% mini agarose gel (after transportation)

1. Prepare gel tray and comb.
2. Weigh out 0.5 g of agarose
3. Add it to 50 ml x TBE flask with a holed lid.
4. Boil gently in a microwave for about 1 min to dissolve the agarose and cool to 50-60°C in the cooling cabinet.
5. Add 2.5µl of ethidium bromide and swirl to mix.
6. Pour the warm agarose gel in a gel tray. Insert the comb and double check that it is correctly positioned.
7. Leave it for about 20 min to set. When set place in gel tank containing 1 x TBE buffer to the point of maximum fill.
8. Prepare samples and controls of five Lambda DNA size standards (6.25; 12.5; 25; 50 and 100 ng/ µl) to be run alongside the samples to quantify extracted DNA samples.
9. Gently remove the comb from the gel tray.
10. Carefully load 5 µl of each sample mixed with loading dye (1 – 2 µl of loading dye).
11. Run gel at 50 V for 15 minutes. Then take gel out from buffer and photograph in the dark room using UV light to see the quantity of DNA samples.

Appendix VII AFLP Protocol

AFLP molecular marker technique is a method used for genotyping individuals for a large number of loci using a minimal number of PCR reactions. It is therefore a new technique for DNA fingerprinting introduced 14 years ago (Vos *et al.*, 1995). AFLP technique is comprised into three key steps:

a) In the first step (restriction digestion), the genomic DNA is digested with two restriction enzymes whereby these two restriction enzymes cut the double-stranded DNA at each restriction enzyme recognition sequence [for *Eco*RI - GAATTC (a 6 base cutter)] and [for *Mse*I - AATT (a 4 base cutter)], generating hundreds thousands of DNA fragments with sticky ends onto which double-stranded adapters are ligated.

b) In the second step, pre-selective amplification PCR is performed using two primers (universal primers) which correspond to the sequence of the adapters and the enzyme plus one additional base. These primers have a two base overhang. Each combination (two bases on the *Mse*I (*Tru*9I) adapter and two bases on the *Eco*RI adapter) reduces the number of DNA fragments.

c) In the third step, selective amplification PCR is performed using two primers with three base overhangs. For any given pre-selective amplification, there are 16 possible selective primer combinations that can be used. The *Eco*RI primer is labelled so that only fragments that contain an *Eco*RI site will be detected and banding patterns (peaks) are then visualized with an automatic sequencer.

1. Preparation

Prepare working concentrations of primes and adaptors

Prepare adaptors by heating to 90°C and cooling slowly to room temperature

Quantify DNA and calculate volume that contains 0.5µg (500ng) DNA e.g for 100 ng/µl use 5µl.

2. Restriction Digestion

Digestion master mix

Component	**Concentration per total number of samples**	**Volume per sample**
Buffer W	10 x	4.0µl
*Eco*RI	50U/µl (5 units)	0.1µl
*Mse*I/ *Tru*9I	(50U/µl)/(10U/µl)(5 units)	0.5µl
PCR grade water		35.4 µl
Total volume		**40.0 µl**

Prepare digestion master mix as above.

Add the appropriate volume of DNA (0.5 µg DNA).

Incubate for 1 hour at 37°C.

3. Ligation Step

Component	**Concentration**	**Volume per sample**
*Eco*RI adaptor	5pMol/µl	1.0µl
*Mse*I /*Tru*9I adaptor	5pMol/µl	1.0µl
T4 DNA Ligase Buffer	10x	1.0µl
T4 DNA Ligase Buffer	1unit	0.3µl
PCR grade water		6.7µl
Total volume		**10.0 µl**

Prepare ligation master mix as above.

Add 10µl of ligation step to each restricted DNA sample.

Incubate tubes in thermal cycler at 37°C for 3 hours.

4. Pre-amplification PCR

Primer mix

Component	Concentration	Volume per sample
Universal primer (E00 for EcoRI)	50 ng/µl	0.6µl
Universal primer (M00 for MseI)	50 ng/µl	0.6µl
PCR grade water		3.8µl
Total volume		**5.0 µl**

Pre-amplification PCR mix to each reaction tube

5 µl DNA from the R/L reaction
5 µl primer mix
10 µl ABgene ReddyMix PCR Master Mix with 3.0 Mm $MgCl_2$

Use thermal cycler (PCR machine) programmed as follows
94 ^{0}C 30 sec}

56 ^{0}C 1min} x 30 cycles

72 ^{0}C 1min}

72 ^{0}C 10min

Check pre-amp product 1% agarose gel containing 1 x TBE buffer for smear.

Use 1 kb ladder.

Photograph gel product using UV-trans-lumination.

5. Selective amplification PRC

Primer Mix for next step

Component	Concentration	Volume per sample
Labeled primer	50ng/µl)	0.5µl
Unlabelled primer (M00 for MseI)	50ng/µl	0.6µl
PCR grade water		3.9µl
		Total 5.0 µl

Prepare selective amplification primer mix as above.

Dilute the pre-amplification PRC product in 1/20 (that is 5µl pre-amplification PRC product into 95 µl of PCR / DNA grade water, which would be 100 µl of diluted pre-amplified products).

PCR Mix

5 µl DNA (from diluted pre-amplification)
5 µl primer mix
10 µl ABgene ReddyMix PRC Master Mix with 3.0 Mm $MgCl_2$

Use thermal cycler (PRC machine) programmed as follows
94 ^{0}C 30 sec}

65 ^{0}C 30secs subtract 0.7 ^{0}C per cycle} x 13

72 ^{0}C 1min}

94 ^{0}C 30 sec}

56 ^{0}C 30 sec}

72 ^{0}C 1min} x 23

10 ^{0}C hold

6. Preparation of PCR products for running on Beckman Coulter CEQ 8000.

PCR products were optimized by loading undiluted samples for fragment analysis. A minimum of one complete row of 8 samples per sample plate was run and if it happens that one row can not be filled up, then 40 µl of SLS were used to fill each empty well.

Component	**Volume per sample**
SLS (de-ionized formamide from Beckman Coulter)	40 µl
Size standard (from Beckman Coulter) per sample well	0.5 µl
Undiluted PCR product per sample well	0.5 µl

Mix SLS with size standard (SS) as above.
Load 40 µl of aliquot (i.e SLS plus SS) in each sample well of sample plate (fill empty sample wells with 40 µl of SLS when needed).
Add 0.5 µl of undiluted PCR products.

Add 2 -3 drops of mineral oil

Prepare and load 1/3 (5 – 6 drops) of separation buffer into buffer plate, in corresponding wells.

The volume was adjusted according to signal intensity of size standards compared to PCR product peaks. There are two forms of size standards available, 400bp (standard fragments range from 60bp to 400bp) and 600bp (standard fragments range from 60bp to 600bp).

Appendix VIII Restriction digestion for all samples

Sample number	Estimated DNA concentration (ng/µl)	Master mix (buffer W, *Eco*RI & *Tru*9I) (µl)	Volume of DNA per sample (µl)	Volume of PCR grade water (µl)	Total volume of each sample (µl)
1	25	4.6	20.0	15.4	40.0
2	25	4.6	20.0	15.4	40.0
3r	50	4.6	10.0	25.4	40.0
4	50	4.6	10.0	25.4	40.0
6	100	4.6	5.0	30.4	40.0
7	6.25	4.6	35.4	0.0	40.0
8r	25	4.6	20.0	15.4	40.0
11r	100	4.6	5.0	30.4	40.0
13	50	4.6	10.0	25.4	40.0
14r	100	4.6	5.0	30.4	40.0
16	25	4.6	20.0	15.4	40.0
17r	50	4.6	10.0	25.4	40.0
19	25	4.6	20.0	15.4	40.0
20	6.25	4.6	35.4	0.0	40.0
21	25	4.6	20.0	15.4	40.0
23	6.25	4.6	35.4	0.0	40.0
24	50	4.6	10.0	25.4	40.0
29r	100	4.6	5.0	30.4	40.0
30	25	4.6	20.0	15.4	40.0
31	25	4.6	20.0	15.4	40.0
32	50	4.6	10.0	25.4	40.0
33	100	4.6	5.0	30.4	40.0
34	25	4.6	20.0	15.4	40.0
35	50	4.6	10.0	25.4	40.0
36r	100	4.6	5.0	30.4	40.0
37	50	4.6	10.0	25.4	40.0
38r	50	4.6	10.0	25.4	40.0
40	6.25	4.6	35.4	0.0	40.0
41r	100	4.6	5.0	30.4	40.0
42r	100	4.6	5.0	30.4	40.0
43	50	4.6	10.0	25.4	40.0
44	50	4.6	10.0	25.4	40.0
45	50	4.6	10.0	25.4	40.0
46	25	4.6	20.0	15.4	40.0
47	100	4.6	5.0	30.4	40.0
48	50	4.6	10.0	25.4	40.0
49	12.5	4.6	25.0	10.4	40.0
50r	50	4.6	10.0	25.4	40.0
52	6.25	4.6	35.4	0.0	40.0
53	25	4.6	20.0	15.4	40.0
55	12.5	4.6	25.0	10.4	40.0
58	6.25	4.6	35.4	0.0	40.0
61	6.25	4.6	35.4	0.0	40.0

62	50	4.6	10.0.	25.4	40.0
63	50	4.6	10.0	25.4	40.0
64	6.25	4.6	35.4	0.0	40.0
65r	100	4.6	5.0	30.4	40.0
66	50	4.6	10.0	25.4	40.0
67	25	4.6	20.0	15.4	40.0
68r	50	4.6	10.0	25.4	40.0
70	6.25	4.6	35.4	0.0	40.0
71	100	4.6	5.0	30.4	40.0
72	50	4.6	10.0	25.4	40.0
73	25	4.6	20.0	15.4	40.0
74r	100	4.6	5.0	30.4	40.0
75	100	4.6	5.0	30.4	40.0
76	25	4.6	20.0	15.4	40.0
80	25	4.6	20.0	15.4	40.0
81	50	4.6	10.0	25.4	40.0
82	50	4.6	10.0	25.4	40.0
83	100	4.6	5.0	30.4	40.0
84	100	4.6	5.0	30.4	40.0
V1	6.25	4.6	35.4	0.0	40.0
V2	6.25	4.6	35.4	0.0	40.0
V3	6.25	4.6	35.4	0.0	40.0
V4	6.25	4.6	35.4	0.0	40.0

Keys: r = repeated DNA extraction sample, V = *A.vera*, 1-84 = *A.zebrina*

Appendix VIIII Protocol for selective amplified PCR product optimization, and sample preparation for running Beckman Coulter CEQ 8000 for fragment analysis.

1. For diluting samples: Pipet 5 µl of SLS into each reaction tube, mix SLS with 2.5 µl of selective amplified PCR products.
    a. Pipet 40 µl of SLS into each sample well.
    b. Pipet 1 µl of 400bp size standard.
    c. Pipet 0.5 µl of diluted selective amplified PCR products to each sample well.
    d. Add two drops of mineral oil
2. For undiluted selective amplified PCR products: The same loading procedure as explained in (1 a – d) above, except that in c) the amount of selective amplified PCR products must be undiluted.
3. Set up separation buffer plate by putting 1/3 of buffer in each buffer plate well. Notice: it must be noted that loaded rows in buffer plates are corresponding with loaded rows for sample well plate.