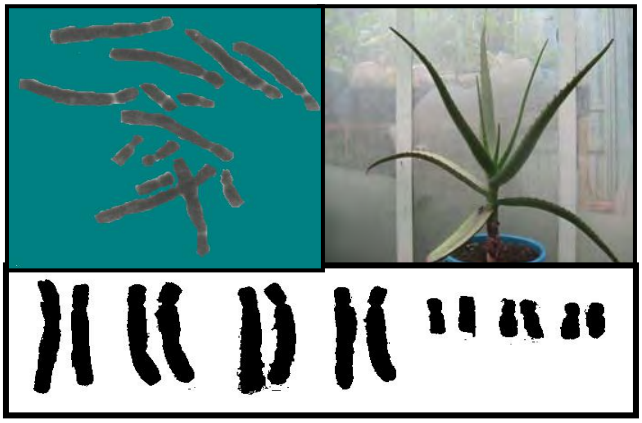
ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTEMENT OF BIOLOGY

CHROMOSOME STUDY ON SEVEN ENDEMIC *ALOE* SPECIES (ALOACEAE) OF ETHIOPIA



A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (Applied Genetics)

BY
ALEMAYEHU MEKURIA

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Declaration

I, the undersigned, declare that this thesis is my original work. It has never been presented for a degree in any other institution and that all sources of material used in it have been duly acknowledged.

Name: _____

Signature: _____

Date: _____

This thesis has been submitted for examination with our approval as university advisors.

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Kifle Dagne (Dr)

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Sebsebe Demissew (prof.)

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Abstract

Seven endemic *Aloe* species, five of which were collected from their respective geographical locations in Ethiopia and two species obtained from the *Aloe* Garden at the Sciense Faculty, Addis Ababa University were studied. They include *Aloe kefaenisis, Aloe harlana, Aloe trigonantha, Aloe yavellana, Aloe debrana, Aloe schelpei,* an *Aloe* (not yet described) species from Bale. Chromosome preparations were made from root tip cells after pretreatment with colchicines or 8- hydroxyquinoline or cold treatment in ice water. Maceration was done in 4 % cellulase + 4 % pectinase solution at 37 ^{0}C for about 1 hr which was followed by air dry slide preparation. Chromosomes were stained with Giemsa in Sorensen's phosphate buffer (PH= 6.8). Root tips were hydrolyzed with 1N HCl for about 10 minutes at 60 ^{0}C in a water bath, the chromosomes were stained either in toluidine blue or aceto–orcein which was followed by squash preparation. The result showed that all species have 2n=14, and one plant of *Aloe schelpei* have 2n = 14 + 1, the additional chromosome being a small metacentric element. The number of satellites observed in different species was varied from one to four. All species showed bimodal karyotype which consists of four pairs of large chromosome (18.26 – 13.82 μm long) and three pairs of small chromosomes (4.11 – 6.00 μm long). The classification of the chromosomes according to centromere position, showed subterminal (st) for the large chromosomes; submedian (sm) and median (m) for the small chromosomes. In the present study no significant chromosomal differentiation in size and morphology between the *Aloe* species investigated were observed. As a result it is not possible to infer species relationships on the basis of chromosome cytology. It was recommended that future cytological studies in *Aloe* should include meiotic analysis in interspecific hybrids and molecular cytogenetic techniques such as FISH and GISH.

Keywords: *Aloe*, Endemic species, Chromosome number, Karyotype.

1. Introduction

1.1.Taxonomic Position of *Aloe*

The genus *Aloe* and related genera were placed in Class Hexandria, Order Monogynia, due to the 6 stamens and a single pistil (Linnaeus, 1753). Subsequently, they have been treated as one of the 28 tribes, namely Aloeae in the family Liliaceae. Later they were included in the alooid genera, along with *Kniphofia* Moench, in the family Aloaceae. Then, the alooid genera were classified into the sub-family Alooidaeae in the family Asphodelaceae. The currently widely accepted position of the alooid plants is in a separate family the Aloaceae, with seven genera: *Aloe* L., *Gasteria* Duval, *Haworthia* Duval, *Lomatophyllum* Willd., *Chortolirion* A. Berger, *Poellnitzia* Uitew., and *Astroloba* Uitew (Smith and van Wyk, 1991; Smith and Steyn, 2004; Fikre Dessalegn, 2006).

1.2. Origin and Geographic Distribution of *Aloe*

Aloe L. (Aloaceae) is a relatively large genus of ~ 400 species which are found from the southern tip of Africa to the Arabian Peninsula, and also on Madagascar and Socotra, and now in the New World where it was introduced at various times over the past four thousand or so years (Holland, 1978; Adams *et al*, 2002; Newton, 2004). Aloes grow in areas rainfall between 200 and 800 m.m. per annum (Holland, 1978).

The center of origin for the genus is suggested to be in the highlands of SE Africa, some time before the land connection with Madagascar was severed in the late Mesozoic to early Tertiary. From there, they spread along the rising highlands of eastern and southern Africa, reaching the Arabian Peninsula by about later part of the Tertiary (Holland, 1978). Holland (1978) further pointed out that, there are eleven secondary speciations recognized in the Highland Africa; among which it is likely that parts of Ethiopian region have become important centers of speciation.

Many countries have some endemic species of *Aloe*. The highest rate of endemism is found in Madagascar and isolated Indian Ocean Islands, and followed by South Africa, Ethiopia, Somalia, Kenya and others (Table 1) (Sebsebe Demissew *et al* 2003; Newton, 2004).

Some species have very wide distribution. *A. buettneri* is the most widely distributed species, with a range of at least 5,600 km, from Mali to Zambia. Another very widely spread species is *A. myriacantha* with a range of about 4,800 km from Kenya and Uganda to the Republic of South Africa (Newton, 2004).

The long-cultivated *Aloe vera* (L) Burm. has become naturalized in many tropical and sub-tropical countries, including some in the New World. The exact origin of *A.vera* is uncertain, but it seems likely that it is the Arabian Peninsula, home of the closely related, and possibly conspecifics, *A. officinalis* Forssk (Newton, 2004).

Table 1. Total number and number of endemic species of *Aloe* and % of endemism by country in Africa (Newton, 2004).

Country	Total number of species	Number of Endemic species	Endemism (%)
Angola	24	13	54.2
Comoros	1	1	100
Democratic Republic of Congo	13	3	23
4Eritrea	8	2	25
Ethiopia	32	26	81.25
Kenya	55	24	43.6
Lesotho	8	1	12.5
Madagascar	75	75	100
Malawi	17	2	11.8
Mauritius	2	2	100
Mozambique	25	3	12
Somalia	33	25	75.8
South Africa	119	71	59.7
Sudan	12	3	25
Swaziland	18	1	5.6
Tanzania	40	13	32.5
Uganda	16	2	12.5
Zambia	19	2	10.5
Zimbabwe	27	5	18.5

1.2.1. Geographical Distribution and Classification of Endemic *Aloe* Species in Ethiopia

The majority of *Aloe* species found in Ethiopia are endemic or near endemic (i.e. their distribution is restricted in one or a few neighboring countries).The endemic Ethiopian and Eritrean taxa fall more or less into three main geographical areas in Ethiopia and Eritrea. The first groups of 14 endemics (two of which are endemic species found in Eritrea, *Aloe eumassawana* Carter, Gilbert and Sebsebe, and *Aloe schoelleri* Schweinfurth) are restricted to the northern and central highlands, North West of the Rift Valley in Ethiopia; the second group includes 5 spp., which are restricted to the eastern highlands, and the third group of 9 species belongs in the south (Table 2) Sebsebe Demissew *et al*., (2003).

Table 2. Endemic or near endemic *Aloe* species in Ethiopia and their geographic distribution (Source Sebsebe Demissew *et al*, 2003)

Species	Distribu-tion (Group)	Species	Distribut-ion (Group)	Species	Distributi-on (Group)
A. digratana	Group 1	*Aloe schelpei*	Group 1	*A. citrina*	Group 3
A .ankoberensis	Group 1	*A. sinana*	Group 1	*A. ellenbeckii*	Group 3
A. camperi	Group 1	*A. steudneri*	Group 1	*A. firsii*	Group 3
A. debrana	Group 1	*A. trigonantha*	Group 1	*A. gilbertii*	Group 3
A. elegans	Group 1	*A. mcloughlinii*	Group 2	*A. jacksonii*	Group 3
A. kefaensis	Group 1	*A. megalacantha*	Group 2	*A. otallensis*	Group 3
A. monticola	Group 1	*A. retrospiciens*	Group 2	*A. rivae*	Group 3
A. percrassa	Group 1	*A. tewoldei*	Group 2	*A. calidophila*	Group 3
A. pulcherrima	Group 1	*A. harlana*	Group 2	*A. yavellana*	Group 3

Group 1:- Northern and central highlands, North West of the Rift valley.
Group 2:- Eastern highlands.
Group 3 :- South Ethiopia.

The following endemic *Aloe* species are included in the present study:

1.2.1.1. *Aloe kefaenisis* M.G.Gilbert & Sebsebe Demissew

Aloe kefaenisis grows in wooded grassland of Keffa region at altitude of above 1800 m. It is one of the most widely cultivated aloes in Addis Ababa and Jimma as an ornamental plant. The species is distinguished from other related species mainly by its less fleshy leaves; the spots which are found on the leaves are much sparser or even absent and in addition to the above features, the basal swelling of the perianth is less distinctly globose (Gilbert and Sebsebe Demissew 1996; Sebsebe Demissew and Gilbert, 1997; Sebsebe Demissew *et al*., 2003).

1.2.1.2. *Aloe harlana* Reynolds

Aloe harlana falls in the second group of *Aloe* species. It grows on sparsely vegetated slopes, often on limestone, within 1650-2400 m altitude in Harerge region (Sebsebe Demissew and Gilbert, 1997). As explained in the Flora of Ethiopia and Eritrea (Sebsebe Demissew and Gilbert, 1997; Sebsebe Demissew et *al*., 2003), the species can be recognized by its brownish tissue along the leaf margins which forms a continuous edge between the spines and by its yellow to red perianth.

1.2.1.3. *Aloe trigonantha* Leach

Aloe trigonantha Leach, fall in the first group of *Aloe*, is found in Gojam and Gondar regions and it grows on dry stony ground near roads and along field margin at altitude between 1900 and 2100m. Regarding its description, as described in the Flora of Ethiopia and Eritrea (Sebsebe Demissew and Gilbert, 1997), it has secondary branching up to 50

racemes or more, leaves in dense rosette which have uniform green color, usually it is stemless, however, some old plants may develop thick and prostrate stems. This species has three-angled perianth with a truncate base which helps to distinguish the spp from the other related group.

1.2.1.4. *Aloe yavellana* Reynolds

Aloe yavellana was described in 1954 by Reynolds, which falls in the third group, in which the species is characterized by succulent shrub, ascending or sprawling stems and it is distinguished from the related species by its cylindrical trigonous perianth. The species grows on rocky slopes in clearings in Juniperous forest, and also in more disturbed areas near roads between 1600 and 1900 m; in Ethiopia it is found around Yavello and Mega (Sebsebe Demissew and Gilbert, 1997; Sebsebe Demissew *et al*., 2003).

1.2.1.5. *Aloe debrana* Christian

A. debrana Christian commonly grows in areas of grassland on thin soil overlying basalt, usually on gentle slopes between 2000 and 2700m above sea level in Shewa, Gojam and Wello regions. It is so far not known from anywhere else (Sebsebe and Gilbert, 1997; Sebsebe Demissew *et al*., 2003). Reynold treated *A. debrana* as a synonym of *A. percrassa*, presumably, on the basis of similar small flowers (Gilbert and Sebsebe Demissew, 1996).

However, Gilbert and Sebsebe Demissew, (1996), Sebsebe Demissew and Gilbert, (1997), Sebsebe Demissew *et al*., (2003), elucidated that the Shewa material collected by Reynolds should be treated under *A. debrana* by giving the following key features. Succulent herb, suckering from base to form small groups, mostly stemless but some old plants develop thick, prostrate stems. Leaves in a very dense rosette, spreading-recurved, 25-60×7.5-15 cm, dull green, old leaves brown when drying.

1.2.1.6. *Aloe schelpei* Reynolds

Aloe schelpei belongs to a group of caulescent aloes mainly characterized by erect, ascending or sprawling stems. The species grows in more open areas within evergreen bushland on steep slopes and cliffs of basalt between altitude of 1700 and 2470m in Shewa region. It is so far not known anywhere else (Sebsebe Demissew and Gilbert, 1997; Sebsebe et *al*., 2003). *Aloe schelpei* is distinguished from the related species by the cylindrical perianth, 27-30 mm long, with the outer segments free for 12-15 mm, the (10-) 12-17 mm long pedicles and the triangular-ovate bracts 6-8 ×2-4 mm.

1.2.1.7. *Aloe* species from Bale

Reynolds (1966) in his book, *The Aloes of Tropical Africa and Madagascar*, emphasized that there are still several new *Aloe* species to be described in Africa; Ethiopia being one of them. After his publication, several *Aloe* species have been discovered. Even after the publication of FEE (Flora of Ethiopia and Eritrea) (1997), two new endemic *Aloe* species (*A. bertemariae* Sebsebe and Doli and *A. friisii* Sebsebe and Gilbert) were discovered (Sebsebe Demissew *et al*., 2003). More recently, a new endemic and yet undescribed *Aloe* species has been added to the collection at the National Herbarium (ETH) (Sebsebe Demissew, Personal communication). This species is 40-70 cm long, growing in clumps with 8-10 stems and it has 10-20 mm diameter, it has also 1-2 inflorescence per stem in which the leaves are spreading along the stem, the flowers secund and perianth is red. This species, found in Bale region, grows at altitude between 1550-1600 m (Sebsebe Demissew, Personal communication).

1.3. Botanical Description of the Genus *Aloe*

According to Sebsebe Demissew *et al*. (2003), the *Aloe* plants are easily recognized by their rosettes of large, thick, succulent leaves, which are sometimes spotted. The rosettes are situated on the ground or on trunks up to 2m. In rare cases, the thick leaves are spaced

along a stem rather than crowded in a rosette. In most species, the leaves are D-shaped in cross section, but some have leaves more or less V-shaped in cross section.

Sebsebe Demissew *et al*. (2003) further elaborated that the leaf margin is almost always armed with sharp teeth. The inflorescence is usually branched (occasionally simple), the lower branches sometimes branching again. Each flower is supported by a bract, the shape and size of which are important for the identification of the species. Flower colouration is most often red, orange or yellow, rarely white. The tepals are fused to form a tube. The upper parts of the tepals are more or less reflexed. The 3+3 stamens are free, inserted at the base of the ovary, exserted in the flowering stage. The capsule wall is papery or slightly woody when mature. The seeds are irregularly 3- sided to flattened, narrowly to broadly winged.

1.3.1. Reproduction Mechanism

Flowers of almost all *Aloe* species are tubular, brightly colored red or yellow, unscented and produce abundant nectar. Almost all aloes are self-incompatible, though oddly enough the flowers are protandrous (anthers dehisce and pollen is dispersed before the stigma is receptive) and as a result selfpollination would not occur anyway (Smith and van Wyk, 1991).

Most *Aloe* species have bright orange or yellow, dorsifixed, oblong to liner-oblong anther with introrse dehiscence. It is hypothesized that flowers with included anthers can be effectively pollinated once a potential pollinater has actively forced its feeding organ into fairly narrow perianth tube to reach the nectar reward (Smith and van Wyk, 1991).

In the case of most species of *Aloe*, they do have stiff erect capsules which are borne on relatively tall inflorescences, and it only open in the upper part. This phenomenon hampers an easy fall out of the seed; and in order to release or eject the seeds, a strong wind or a strong kick is required which suggests an adaptation to wind dispersal of seed (anemochory)

(Smith and van Wyk, 1991). The flowers of *Aloe* vertically disposed on more or less horizontal inflorescence axes. This appears to be an adaptation to bird pollination, the usually robust, horizontal peduncles acting as perches for avian visitors (Smith and van Wyk, 1991).

Some species of *Aloe* are arborescent, and others are virtually herbaceous, but all tend to be shallow rooted, long-lived, succulent plants which flower during the winter months, produce large number of small air-borne seeds, and propagated sexually (Holland , 1978). In an area where two or more species of *Aloe* flower at the same time, the main pollen vectors, sunbirds, fly indiscriminately from one species to another. Consequently hybridization is frequent (Smith and van Wyk, 1991).

As suggested by Sebsebe Demissew *et al*. (2003), in addition to reproduction by seed, several species have the ability to produce lateral rosettes that may develop into independent individuals by fragmentation (suckering). Vegetative propagation might be an important factor for maintaining the population. This is often seen in the spatial arrangement of individuals, which occur in cluster (Sebsebe Demissew *et al*., 2003; Fikre Dessalegn, 2006). Species from Madagascar (*A. bulbillifera* H. Perrier) and Congo (A. *patersonii* B. Mathew) have regular occurrence of bulbil formation. The bulbils are formed in the axils of inflorescence branches. This phenomenon was also observed once on a cultivated plant of the Kenyan *A. lateritia* (Newton, 2004; Fikre Dessalegn, 2006).

1.3.2. Ecology of *Aloe* species

Members of the genus *Aloe* are ecologically heterogeneous with a wide range of distribution and has diversified into almost every possible habitat, ranging from desert shrublands, close-canopy, dry forests, grassland, exposed rock surface, cliff faces and savanna to comparatively high rainfall coastal forest types (Holland, 1978; Smith and van Wyk, 1991; Newton, 2004). But, according to Newton (2004), they are absent from the

moist lowland forests of mainland Africa, however, they are found in the dry coastal forests of E. Africa including arborescent species.

Many shrubby species are found in *Acacia* scrub and other thickets. On the other hand, grassland offers a habitat for many acaulescent species. *Aloe* species occupy a considerable altitudinal range; they may be found from sea level (e.g. *A. boscawenii* Christian, *Aloe eumassawana* Carter, *A. kilifiensis* Christian) up to about 3500 m (e.g. *A. ankoberensis* Gilbert and Sebsebe)(Newton, 2004).

1.4. Economic and Medicinal Importance of ***Aloe*** Species

1.4.1. **Pharmacology, therapeutic applications and other uses of *Aloe***

The word aloe is from the Arabic *alloeh* or the Hebrew *halal* and it means a shinning, bitter substance (Mascolo *et al*., 2004). The characters that unify members of the Aloaceae are the presence of 1-methyl 8-hydroxyanthraquinones in the roots and anthrone-c-glucoside in the leaves, and vascular bundle containing a parenchymatous inner bundle sheath (Smith and steyn, 2004).

Most species of *Aloe* have a group of cells associated with the vascular bundles, variously called aloin or alloitic cells that store and perhaps secrete a mixture of compounds with medicinal value, with the composition varying in different species (Newton, 2004). Out of the wide range of *Aloe* species, only *A. ferox* and *A.vera* are of importance in international trade. However, it remains to establish if extracts from other spp. could be promoted for similar purpose (Erimias Dagne *et al*., 2000).

Aloe has been used as medicinal plants for centuries. Three distinct preparations of aloe plants are mostly used in a medicinal capacity: aloe latex, aloe gel, and whole leaf (aloe

extract). Aloe latex is used for its laxative effect; aloe gel, is used topically for skin ointments; and aloe extract is potentially used for cancer (Mascolo *et al*., 2004).

Aloe vera (L) Burm.f. has enjoyed a long history as a medicinal plant. The leaf of *A. vera* consists of two parts, the inner clear pulp and the outer green rind. Many of the beneficial effects of this plant have been attributed to the pulp, which has both immuno-stimulation and anti-inflammation effects (Ni and Tizard, 2004).

The leaves of *A. vera* are used in the production of many cosmetics; due to the softening properties and anti-ageing effects on the skin products (Mascolo *et al*., 2004). The many kinds of products on the market include after shaving gel, hair tonic, shampoo and skin moistening gel (Farnsworth and Bunyapraphatsara, 1992; Newton, 2004). *Aloe* gel possesses anti-inflammatory and immunodulatory properties and it serves as a stimulant for wound healing, a fuel for proliferating cells and dressing for open wounds (Farnsworth and Bunyapraphatsara, 1992).

Clinical trials are now in progress to provide conclusive evidence in diseases such as arthritis, gastric ulcer, cancer, AIDS and colitis (Mascolo *et al*., 2004). Some other uses are also based on the chemical content. An insect repellent can be made by drying and burning aloe leaves and protect stored food against weevils (Newton, 2004).

In South Africa, the leaf sap of *A. maculata* Baker and *A. confuse Engl*. are used to dye cloth and making ink (Newton, 2004). In many African countries aloes are used in gardens as decorative plants (e.g. in E. Africa, *A. dawei* Berger and *A. kedongensis* Reynolds are used as hedge). In Kenya, *A. chrysostachys* is planted in rows on eroded slopes in an attempt to protect the soil erosion (Newton, 2004).

1.4.2. Traditional Medicine

Aloes have been used as a common folk medicine in several countries (Sebsebe Demissew *et al*., 2003; Mascolo *et al*., 2004*)*. The whole plant has been used in India to treat stomachache and as anti-helimintic; the leaf pulp has also been used for menstrual suppression and the root for stomach pain. In China, it is a common dermatological remedy and in Mexico it is used to treat minor skin irritations (Mascolo *et al*., 2004). Furthermore, according to Dawit Abebe and Ahadu Ayehu (1993), in northern part of Ethiopia, people use *Aloe* species as a traditional medicine to treat urinary retention, cataract, rectal prolepses, ascaris, infertility and coughs. The pharmacological activities and clinical trials on *A. vera* and *A. ferox* revealed some of the above uses (Farnsworth and Bunyapraphatsara, 1992). Particularly, the solidified exudates which are obtained by evaporating the liquid that drains from the transverse leaves of the various species of *Aloe* are used as dermatological agent even if the official drug is mainly obtained from *A. ferox* and *A. vera* (Dawit Abebe *et al*., 2003). As mentioned on the *Review of Significant Trade East African Aloes* (Anonymous, 2003), it is generally known that almost all *Aloe* species are used medicinally even though their local trade is informal and undocumented.

Aloe has also been used for centuries for a multiplicity of unrelated human illness; for example to correct kidney ailments, to enhance sexual excitement, to develop the mammary glands, to relieve headaches and reduce fever in child (Mascolo *et al*., 2004).

Out of a large number of *Aloe* species, only a few are of importance in international trade with the most outstanding being *A. ferox* and *A. vera* (Erimias Dagne *et al*., 2000). However, neighboring country, Kenya, has earned hard currency by exporting *Aloe* species derivatives, extracts, live plants, leaves and specimens of *Aloe* species to Far East, Middle East, Europe and USA (Anonymous, 2003). On the other hand, so far, little is known about the chemistry of the Ethiopian *Aloe* species and this is what needs further study, because the endemic Ethiopian *Aloe* species represent an economic potential (Sebsebe Demissew *et al*., 2003).

1.5. Conservation

Many species of the genus *Aloe* are regarded as endangered species because of various threats to them which include: over-collection of plants for cultivation which lead to the depletion of well-known wild population, destruction of plants for harvesting leaf exudates and destruction of natural habitats (Newton, 2004).

Newton (2004) indicated that there is a lucrative trade in leaf exudates, mainly required for medicinal and cosmetic purposes, and these are frequently harvested from wild plants. For instance, in South Africa alone, 700 tons of crystalline bitter is harvested each year from about 17 million of *A. ferox* plants, 95% of which are collected from the wild.

One of the problems that lead to destruction of habitat is over grazing. Many people in arid areas have herd of domestic animals in a number far greater than the carrying capacity of the land, and thus the land becomes increasingly denuded of vegetation. In many countries where *Aloes* are native, the rise in human population number resulted in an increased demand for land to use for agriculture and building .This has led to whole scale clearing of natural vegetation. In some areas, the continued expansion of human population is forcing people to move into arid areas, where many aloes occur (Newton, 2004). According to Fikre Dessalegn (2006), in Ethiopia some of *Aloe* spp. encounter major threats ; *A. sinana* and *A. deberana* are threatened due to habitat destruction for the expansion of agricultural land ; *A . Percrassa* due to road construction; and also *A. gilbertii* (the species found near Awassa town) as a result of urbanization and road construction.

1.6. Molecular Study of ***Aloe*** species in Ethiopia

The status of eleven *Aloe* species in Ethiopia was revised based on morphological and molecular (AFLP: Amplified Fragmented Length Polymorphism) analysis (Fikre Dessalegn, 2006). The study showed that nine of the species: *A. harlana* Reynolds, A.

monticola Reynolds. *A. debrana* Christian, *A. percrassa* Tod., *A. yavellana* Reynolds, *A. megalachanta* Baker, *A. gilbertii* Sebsebe and Brandham, *A. calidophila* Reynolds and *A. sinana* Reynolds retained their species status, where as *A. camperi* Schweinfurth and *A. adigratana* Reynolds found to be genetically too closely related to be considered as separate species. Based on this close relationship, combined with close resemblance in morphology and geographical proximity, it was suggested that the two species should be combined as subspecies level as: *A. camperi Schweinfurth* subsp. *camperi* and A. *camperi* susp. *adigratana* (Reynolds) Fikre (Fikre Dessalegn, 2006).

1.7. Cytogenetic Study

Cytological characters, including chromosome number and karyotype analysis, may have been considered as a reliable guide in the studies of taxonomic and evolutionary relationship (Soliman, 2002).When chromosome studies are combined with hybridization and genetic analysis, they provide essential clues in tracing the origin and the evolutionary history of plant species. The number, size and morphology of chromosomes are used to characterize the karyotype of plants and are used to define the taxonomic differences between them (Soliman, 2002).

Although some taxonomists have asserted that chromosomal differences represent just another morphological character and should be treated in the same way as the various characters of external morphology (Stebbins, 1971), there are several reasons why chromosomes are important in taxonomy; they occur in sets of defined number and can be counted during mitotic division, they have defined shape that can be expressed in absolute and relative length units; as a result chromosome characters can be incorporated validly into floristic description and utilized in taxometric and cladistic analyses (Stace, 2000). Furthermore, in order to supplement the conservation programme, it is important to know the structure and behavior of chromosomes and genome to elucidate evolutionary potential of population and also to underpin the structural rearrangement (Lavania, 2002).

1.7.1. Chromosome number

Chromosome counts exist for only ~25% of Angiosperm species (Bennett, 1998; Stace, 2000) and in case of tropical areas it is less than 1 % (Stace, 2000). Even this information is incomplete or incorrect for many species because many counts were made for only one individual or population, and one count may give very incomplete picture for a species (Bennett, 1998).

The study of chromosomes number shows the existence of wide variation in chromosome number in plants. Variations in chromosome numbers are observed even among closely related species. One way of chromosome number alteration is aneuploidy, in which the chromosome number of individual organisms differs from that of the wild type by part of a chromosome set, usually as a result of loss or gain of one or a small number of chromosomes (Raven, 1999; Griffiths *et al*., 2000). Aneuploidy is a by product of unequal translocation between non-homologous chromosomes, or fusion or fission of chromosomes (Stebins, 1971). B- chromosomes also are extra chromosomes to the standard complement and occur in many organisms. They can originate in a number of ways including derivation from autosomes and sex chromosomes in intra and interspecies crosses (Camacho *et al*., 2000). One of the features of many B- chromosomes is that they are absent from some individuals of a population, and when present the number varies between different organisms or tissues of the organisms (Camacho *et al*., 2000).

1.7.1.1. Polyploidy

Another type of chromosomal numerical change is polyploidy in which an organisms possess three or more genomes (chromosome sets) (Jackson, 1971*;* De Wet 1971). It is extremely common in all the major groups of plants and its extent can be revealed only in conjunction with a decision on base numbers. The polyploidy figure of angiosperm ranges

from 35% to 70% out of the chromosome count of 25% of angiosperm species (Stace, 2000).

As indicated by De Wet *(*1971), polyploids arise from chromosome doubling in somatic cells, or through the functioning of cytologically unreduced gametes. They are classified into autoploids and alloploids on the basis of assumed origin. Autoploidy usually refers to the presence of three or more copies of the same genome, while through alloploidy the complete genomes of two well differentiated species are combined in a hybrid (De Wet, 1971)

1.7.1.2. Genome size

Species also differ in terms of their genome size and c-value, which denote the quantity of DNA present in the chromosome complement (Johann, 1988). Only 1% of angiosperm species are with their DNA C-values determined (Bennett, 1998).

Comparative studies of the angiosperms have played a leading role in showing that DNA C-value is correlated with a wide range of phenotypic characters at cellular level (Bennett, 1998).The amount of chromatin per cell varies greatly, and these amounts seem constant for each species (Stace, 2000). C-value vary two or three folds even in closely related species with the same chromosome number (Johann *et al*, 1988).However according to Stebbins (1971), variation in absolute size, including the total DNA content of the nucleus, may vary as much as 20 fold between genera of the same family having the same or similar basic chromosome number. Most of the evidence now points to the conclusion that the general trend in angiosperm evolution is towards higher C-value (Stace, 2000).

1.7.2. Chromosome morphology

Chromosome morphology is usually studied at the metaphase stage in mitosis, when chromosomes have become contracted to the maximum extent or nearly so in their division cycle, and when they are most easily stained (Stebbins, 1971). According to John (1976),

the length of a chromosome is constant and chromosomes may arbitrary be classified as long (>10 μ m), medium (4-8 μ m), or short (>2 μ m). In addition to their sizes, chromosomes are characterized by morphological features of which the important ones are briefly described below:

1.7.2.1. Centromere

The centromere, also known as primary constriction, is a constricted region on a metaphase chromosome and it is due to under condensation in the region relative to the other origins of the chromosome. The chromosomes of most species of eukaryotic organisms are monocentric, i.e. normally possess one functional centromere each. A few group of organisms have holocentrics chromosomes which presumably have many specific centric sites along their length and so no constriction is present (Jackson, 1971).

The most obvious feature defining chromosome morphological variation is the position of the centromere (Stace, 2000). According to the centeromere position, chromosomes are designated as follows (Stebbins, 1971).

Metacentric: - The centromere is located at or near the middle of the chromosome, so that its arms are nearly or quite equal in length (Stebbins, 1971; John, 1976).
Sub-metacentric:- The centromere near to one end of the chromosome than the other, so that the two arms are distinctly unequal, but less so than in acrocentric chromosomes (Stebbins, 1971; Singh, 2003).
Acrocentric: - The centromere is located near one end of the chromosome, so that the chromosome contains one long, and one very short arms (Stebbins, 1971; John, 1976).
Telocentric:- This is where the centromere is strictly terminal entity; and the chromosome is one armed (John, 1976).

1.7. 2. 2. Secondary constriction and satellites

One or a few pairs of chromosomes in the diploid complement possess a constricted region other than the centromere. This is known as a secondary constriction and when present it is located between the centromere (primary constriction) and the end of the chromosome arm (Jackson, 1971). When present, a secondary constriction occupies a constant location and it serves as a morphological marker which gives the chromosome a distinctive morphology (John, 1976).The number of chromosomes that bear secondary constriction range from a single pair to several pairs per diploid genome depending up on the species (John, 1976).

Functionally, secondary constrictions are the sites where DNA that is transcribed into rRNA is located and nucleolus is formed, and thus they are also known as nucleolar organizing regions (NORs) or rDNA sites. Frequently, NORs are subterminal in position (Stace, 2000).

As stated by Stace (2000), satellites are formed by secondary constrictions on the nucleolar organizer chromosomes. These are chromosomal segments separated from the main body of the chromosome by a secondary constriction. The size of satellites can vary considerably between non-homologus chromosomes depending on the relative position of the secondary constrictions on a chromosome. They are also found to be very variable in appearance as they are sometimes very conspicuous and at other times indiscernible and thus it is not always possible to obtain consistent results (Stace, 2000).

1.7.3. Karyotype

Karyotype is defined as the phenotypic appearance of the somatic chromosomes in contrast to their gentic content. In other word, it is the variation among the chromosomes of a genome, which may include features like centromeric position, relative size, absolute size, number and sites of secondary constriction etc (Stace, 2000). Changes in chromosome

number and morphology are often associated with speciation (De Wet, 1971). Stebbins (1971) indicated that the availability of pioneer habitats and environmental extremes are directly or indirectly conducive to chromosomal reorganization. As the result of karyotypic changes in the process of speciation, karyotypes of different species may differ from one another in one or more of the following component of the karyotype such as chromosome number; size of localized centromeres and the number of microtubules attachment sites; arm ratios; numbers, size, and position of secondary constrictions and satellites; size differences within a complement; and position, number, size and distribution of differentially staining heterochromatic segments. This means that karyotypes undergo evolutionary changes to smaller or larger extents in the process of organism speciation.

The evolution of karyotype can be studied by observing and comparing the component of karyotype: For example, differences in absolute chromosome size between related species or genera may reflect different amount of gene duplication (Stebbins, 1971; and Jackson, 1971). Differences in centromeric position, is brought about by pericentric inversion or unequal translocations; differences in relative chromosome size can be brought about by segmental interchange involving translocation of unequal size. Differences in the number and position of satellite reflect differences in the location and size of nucleolar organizer regions (Stebbins, 1971)

Karyotypes are categorized as symmetrical and asymmetrical karyotypes. A symmetrical karyotype is one in which the chromosomes are all of approximately the same size, and have median or sub-median centromeres **(**Stebbins, 1971; Stace, 2000). Where as asymmetrical karyotype consist of chromosomes of unequal sizes with submedian and subterminal centromere. Asymmetry karyotype can occur either through the shift of centeromere position from median to sub terminal or terminal, or through the accumulation of differences in relative size between the chromosomes of the complement, thus making the karyotype more heterogeneous(Stebbins, 1971). It seems that, at least in plants, changes towards asymmetry of the karyotype goes together with speciation. Those organisms which

have homogeneous karyotypes are regarded as more primitive, and heterogeneous karyotypes as more specialized (Stebbins, 1971).

The main mechanisms by which the asymmetry of karyotype increases are pericentric inversions and unequal translocations of portions of chromosome arms. It may, therefore, take place without changing the number of centromeres or of independent chromosomes. Furthermore, by converting metacentric to telocentric chromosomes, pericentric inversions can reduce the fundamental number of chromosome arms (Stebbins, 1971; Johnson, 2003).

A number of asymmetrical karyotypes consists of distinctly large and small classes of chromosomes. Such karyotypes are referred to as bimodal karyotypes. On the other hand, centric fusion, consist of transfer of whole arms between acro- or telocentric chromosomes and increases karyotype symmetry by giving rise to metacentric chromosomes. Consequently, it produces a reduction in the number of centromeres and chromosomes, which leaves the fundamental number of arms unchanged (Stebbins, 1971).Therefore, it is possible to determine which of these process has been chiefly responsible for chromosomal changes by comparing karyotype of related species (Jackson, 1971)

1.7.4. Chromosome cytology of the genus *Aloe*

Cytologically, *Aloe* and its close relatives are remarkable for the uniformity of the basic number and the gross morphology of their chromosomes. Every species of *Aloe* that has been investigated cytologically has bimodal complement, with eight long sub-metacentric to acrocentric chromosomes and six short ones. The gross morphology of the chromosomes is identical in all species and that four long ones in the basic set can be identified easily (Brandham, 1971).The L1 is the longest overall, with the longest short arm length and the L2 and L3 are intermediate, the three short chromosomes can not be distinguished from each other, except in few species, such as *A. tenuior* in which there is a nucleolar organizer on the short arm of one pair (Brandham, 1971; 2004).

All the chromosomes are large compared with those of most angiosperms, the short ones averaging 1.5-3μm in length and the long ones almost 4.6 times larger in a sample of *Aloe* species (Brandham and Doherty, 1998; Brandham, 2004).
Although the basic karyotype is uniform throughout the family, major structural changes in the chromosomes are wide spread , with Robertsonian fusions , large translocation and other types of interchanges, pericentric and parcentric inversions of differing sizes being also common (Brandham and Doherty , 1998).

Polyploidy is uncommon in *Aloe* and is not uniform in its geographical distribution in the genus. In southern Africa, where a large number of *Aloe* species occur, only *A.ciliaris* Haw is known to be a polyploid, 2n = 42 (Brandham, 2004). However, relatively polyploidy is more common among the East African aloes. So far, *A. inermis* Forssk. and *A. cremnophila* Reynolds, *A. juvenna* Brandham and Carter and the Ethiopian *A. jacksonii* Reynolds are known to be tetraploids 2n = 28 (Brandham and Carter,1982). There are a few reports of triploidy in *Aloe* (2n = 3x = 21) (Brandham, 1971).

The origin of the two size groups of chromosome of the bimodal karyotype in *Aloe* has been established. One speculation is that , *Aloe* is an allotetraploid derived from a hybridization between an x = 3 and x = 4 plants followed by chromosome doubling; where it might then be expected that the two original genetically dissimilar genomes (now the long and short chromosomes) would have behaved differently in subsequent evolution, the former increasing in size more rapidly than the short one (Brandham, 1982).The other possible origin of a bimodal karyotype has been unequal translocation, by means of which certain chromosomes would periodically contribute segments to others of the same complement (Stebbins,1971).

Despite the remarkable karyotypic uniformity of the Aloaceae, a preliminary investigation has shown that some variation occurs between species and genera in overall chromosome size and in nuclear DNA amount (Brandham and Doherty, 1998). The 4C DNA values were examined in a sample of 20 *Aloe* species, having a range of evolutionary

advancement and including two tetraploid species. The C- values ranged over two folds in the diploids from 42 – 43 pg in *A. tenuior* and *A. distans* (primitive spp.) to nearly 80 pg in *A. ammophila* and over 95 pg in *A. peckii* (Advanced spp.). The highest values were obtained in tetraploid spp., 116 pg in *A. ngobitensis* and 124 pg in *A. juvenna* (Brandham and Doherty, 1998). Similar trend was also observed by Zonneveld (2002), in which the 2C nuclear DNA content of 83 *Aloe* species, as studied by flow cytometry with propidium iodide, was shown to range from about 16 to 44 pg. There is an indication of marked overall trend towards an increase in nuclear DNA content with evolutionary advancement in *Aloe* (Brandham and Doherty, 1998).

The proportion of long vs short chromosomes of 65 *Aloe* species, investigated by Brandham and Doherty, (1998), indicated that the value lies within a range of 4:1 to 5:1, with the majority being near the midpoint of the range, 4.6:1. This is thus an indication that the evolutionary increment of DNA amount is distributed among the long and short chromosomes in such a way that their relative proportions are retained. Evolutionary chromosome changes in aloes involves an alteration in DNA amount while at the same time it maintains karyotpic orthoselection and preserves the relative proportions of the numbers of the haploid chromosome set (Brandham and Doherty, 1998).

By using FISH, Adams *et al* (2000) investigated the distribution of 5S and 18S-5.8S-26S rDNA sequences in 13 *Aloe* species selected to include species from across geographical ranges and a range of morphological types. They found similar locations of 5S rDNA in all species which indicated that the location is conserved; whereas, in contrast, the location of 18S-5.8S-26S rDNA was highly variable between species.

Regarding the cytology of Ethiopian *Aloe* spp., the Karyotype, C-banding, satellite chromosomes and nucleolar number of A. *ruspolina*, *A. pulcherrima*, *A. citrina*, *A. pirottae, A. trichnosantha* , *A. sinana* have been reported (Mohammed Abate, 2004). The study revealed that they all have the basic chromosome number (x = 7), and bimodal karyotype with a karyotypic formula of 1m + 2sm + 4st; with a maximum number of nucleoli varying

from 4 to 6 in different species which correlates with the number of satellite chromosomes (Mohammed Abate, 2004)

It is important to recognize that the full range of chromosome numbers in Angiosperms is uncertain. So, there is a real need for more basic work (Bennett, 1998). Besides, as pointed out by Stace (2000), a fuller documentation of basic chromosome data is an important priority because of the problems we face in conserving the world's plant genetic resources. It should be remembered that cytological details, unlike anatomical, chemical and molecular data, are lost in preserved data materials. It is with this broad objective in mind that the present study has been undertaken.

2. Objectives of the study

2.1 General objectives

To generate cytogenetic information on seven endemic *Aloe* species in Ethiopia; and to determine cytogenetic similarity and difference among the species.

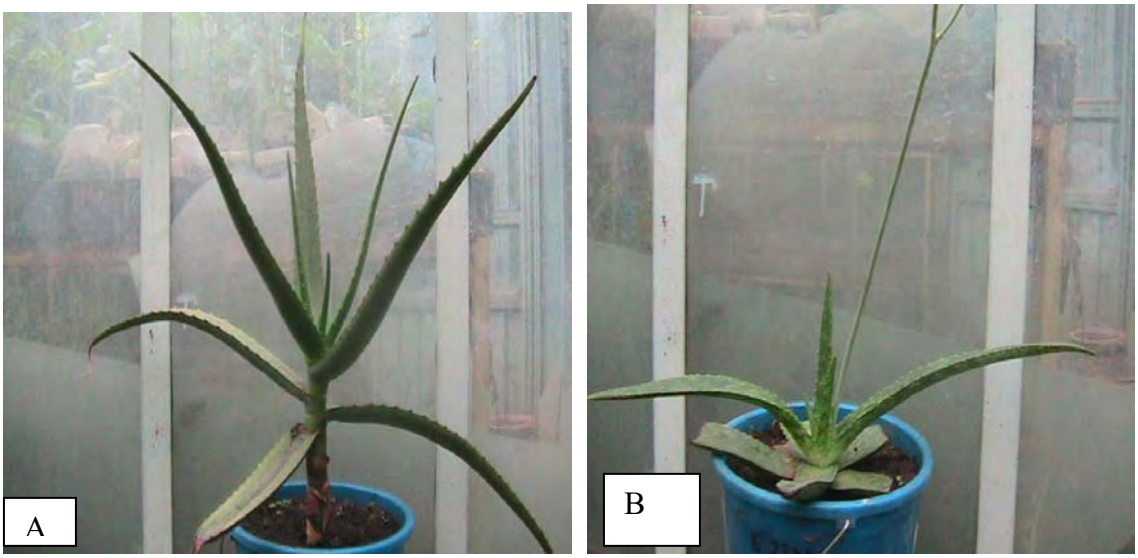
2.1.1. Specific objectives

- To determine the chromosome number of seven endemic *Aloe* species.
- To construct karyotype of the studied species.
- To determine the ploidy level on the basis of basic chromosome number of the studied species.

3. Materials and Methods

3.1. Plant Materials

In the present study, seven *Aloe* species endemic to Ethiopia were studied. These are presented in Table 3 and Fig.1. Two of the species, *A. kefaenisis* and *A. yavellana* (Fig.1 A and B) were obtained from the *Aloe* Garden at Sciense Faculty, Addis Ababa University. The remaining five species (Fig.1, C-G) were collected from their natural habitats and transplanted into pots and grown in the greenhouse at Science Faculty, Addis Ababa University. Fresh root tips of each specimen were used for chromosome study.



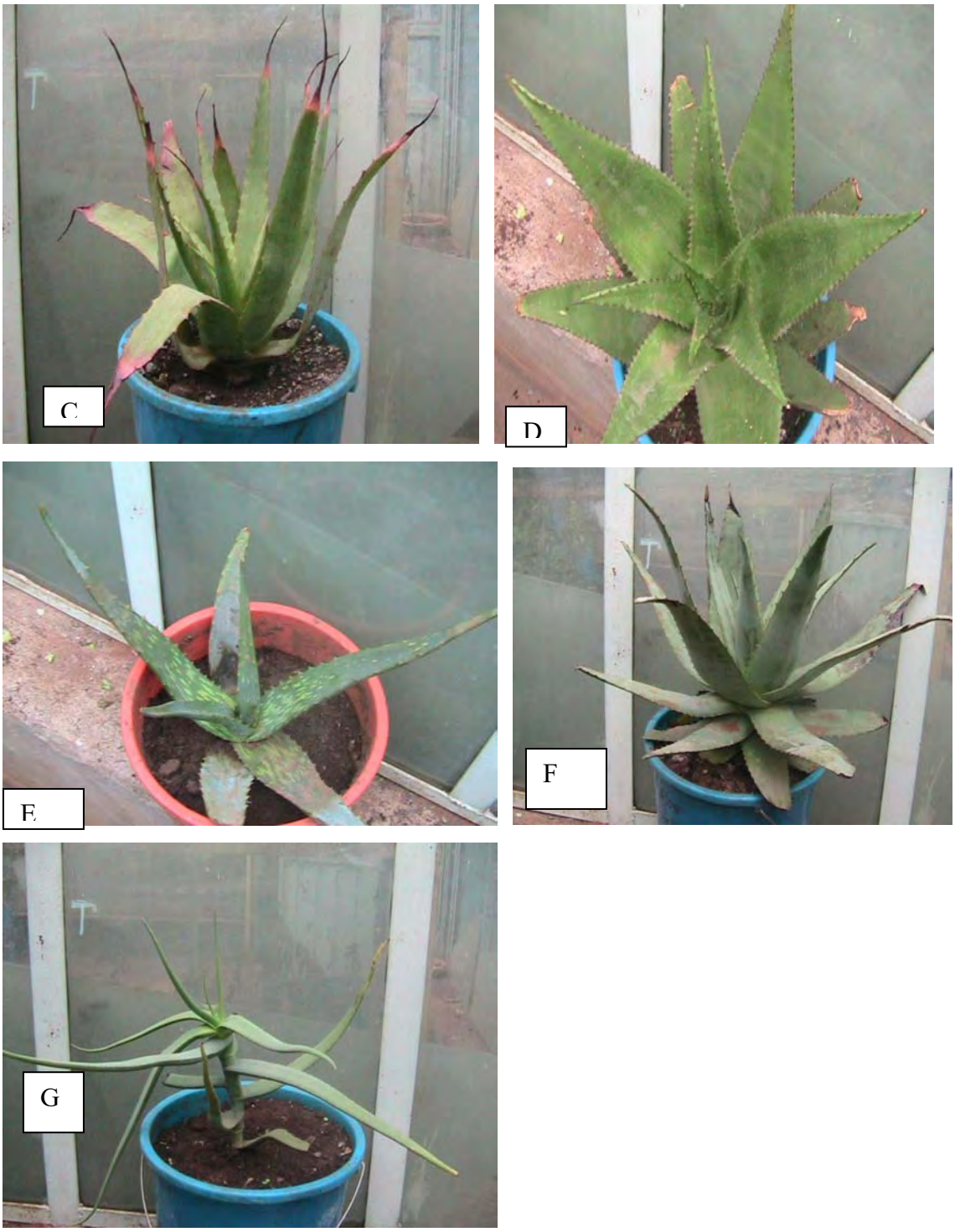


Fig.1. Pictures of *Aloe* species used in the present study: A. *Aloe yavellana*; B. *A. Kefaenisis*; C. *A. trigonantha*; D. *A harlana*; E. *A. schelpei*; F. *A. debrana*; G. *Aloe* species from Bale.

Table 3. List of *Aloe* species studied, sites of collection and their collection number.

Species	Site of specimen collection	Collection number
A. yavellana	AAU, Science Faculty, *Aloe* Garden	AG/05/08
A. kefaenisis	AAU, Science Faculty, *Aloe* Garden	AG /05/08
A. trigonantha	Gondar	HC / 05/09
A. harlana	Hararghe	HC/05/09
Aloe spp	Bale	SD /05/09
A. debrana	North Shoa	HC/05/09
A. schelpei	North Shoa	KD /05/11

3.2. Somatic chromosome analysis

3.2.1. Pretreatment

For somatic chromosome studies, actively growing root tips were harvested from the specimens growing in the greenhouse and collected in vials. The roots were then either cold treated by keeping the vials in ice water for 24 to 26 hours or treated with colchicine (0.1- 0.2 %) or 8-hydroxyquinoline (0.002 M) for 2-4 hours at room temperature. These treatments inhibit spindle formation and result in metaphase arrest, thus facilitating chromosome contraction and spreading (Walker, 1973). The root were then transferred to clean vials and fixed in ethanol acetic acid (3:1, v/v) for 1 hour or more at 4^{o}C. The prime purpose of fixation is to coagulate the cell contents in order to retain their shape, structure and position. It also prepares the surface of the chromosomes so that they readily take up suitable stains (Walker, 1973). Following fixation, the roots were stored in 70% ethanol at 4 °C until used. After brief rinsing of the roots in distilled water, enzyme maceration was performed in 4 % cellulase plus 4% pectinase solution for about 1-2 hour at 36-37^{o}C (Kifle Dagne and Heneen, 1992) or hydrolyze in 1N HCl for ten minute at 60^{o}C (Greilhuber and

Ehrendorfer, 1988; Paton, 1992). The roots were rinsed in several changes of distilled water after decanting the enzyme or HCl solution.

3.2.2. **Slide preparation**

3.2.2.1. Airdry Technique

When the roots are well macerated in pectinace-cellulase solution, the lower tips (1-2 mm) detach from the roots by themselves or upon slight agitation. The macerating enzyme was carefully removed with a Pasture pipette and the roots were rinsed in distilled water. The detached root tips were pipetted onto glass slides. The water was blotted off by carefully touching the edge of the water drop with a piece of filter paper. A drop or two of fresh fixative (3:1, ethanol: acetic acid) were then placed on the root tips, and the root tips were gently mashed with a flat end needle or forceps. Then, cells were spread by strong air blowing on the slide. The slides were then allowed to air-dry under room temperature. The air-dried slides were stained with Giemsa stain in Sorensen's phosphate buffer (pH 6.8) for about 30 minutes or more. Then, the slides were rinsed in distilled water, air-dried and permanently mounted with cover slips using DPX, which is a mixture of Distyrene, Plactcister and Xylene (Gustashaw, 1991).

3.2.2.2. Squash Technique

The root tips which were stored in a refrigerator at 4^{o}C were rinsed and hydrolyzed with 1 N HCl for about 10 minutes at 60 ^{o}C in a water bath. The HCl was removed, and roots were rinsed in distilled water. The root tips were transferred to a watch-glass and stained with toludine blue or aceto-orcein for 20-30 minutes. The merstemic end of the root tips were cut and mashed with a flat end needle in a drop of the stain. Then the root tips were squashed by pressing on the cover slip with the thumb and the slides were made semi-permanent by sealing the edges of the cover slip with paraffin wax or nail varnish. The

preparation was observed under the microscope, and slides with better chromosome preparation were placed in a beaker of absolute ethanol until the coverslip falls off. Both the slides and the coverslip were allowed to air dry and permanently mounted with DPX.

The prepared slides in both techniques were examined for chromosome spreads under light microscope and Photomicrographs of good metaphase chromosome spreads were taken using a camera fitted microscope at a magnification of x1000 (i.e., x10 eyepiece and x100 objective).

3.2.3. Karyotype Analysis

Chromosomes were described and characterized using photomicrographs and direct observation under the microscope. From a set of mitotic metaphase plates of each species, a representative chromosome spread was selected and scanned into computer and the total length and arm lengths of the chromosome, in pixels per cm, were measured using micromeasure computer software version 3.3. Arm ratios of individual chromosomes were calculated by dividing the length of the long arm of the chromosome by that of the short arm. The karyotypes were constructed from pictures of the metaphase plates by cutting and arranging homologous chromosomes into pairs using centromeric position (arm ratio) and chromosome size as criteria.

The actual size of the chromosomes in µm were determined by using a stage micrometer, in which 1mm is divided into 100 parts, each part being 0.01mm. The stage micrometer was photographed at the same magnification as that of the chromosomes and printed at the same enlargement like the chromosome pictures. The enlarged pictures of the chromosomes were then measured with cm rulers and the measurements were converted to µm using the enlarged pictures of the stage micrometer.

Chromosome morphology was determined based on arm ratio (r) as proposed by Levan *et a*l, (1964). Based on this, median point (M) and median region (m) were classified as

metacentric chromosomes, when r = 1.0 - 1.7; submedian region (sm) as submetacentric chromosomes, when, r = 1.7 - 3.0, and subterminal region (st) as subtelocentric chromosomes when r = 3.0 - 7.0

4. Results

Chromosome numbers of the species *A. kefaenisis*, *A. harlana*, *A. trigonantha*, *A. yavellana*, *A. debrana*, *A. schelpei*, and a species of *Aloe* from Bale were studied and the results are presented in Table 4. The chromosome counts were based on maximum counts from more than one intact cell. All the species studied were found to be diploid, (2n = 14), except *A. schelpei*, in which one additional small chromosome was observed in one plant; Table 4 presents the summary of the chromosome counts obtained for the species studied. Table 5 presents the absolute sizes of the chromosomes. These chromosome numbers, except that of *A. harlana* and *Aloe debrana*, are believed to be the first report for the species.

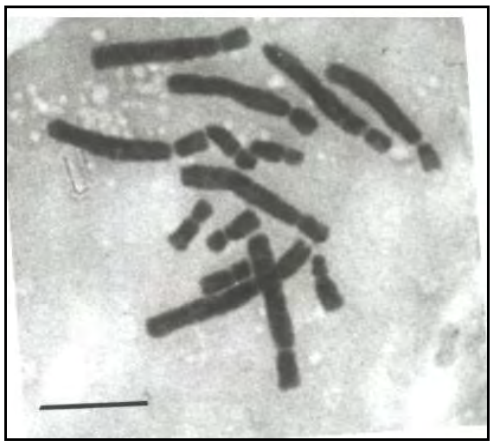
Karyotypic analysis, including chromosome number, arm ratio, and total complement length, are presented (Appendices 1-7). The seven species of *Aloe* studied displayed bimodal karyotype formed by eight large chromosomes, whose length ranged 11.68 – 18.26 μm and six moderately short (S) chromosomes with a range of 4.11 μm to 6.00 μm length. The centromeric position for the long chromosomes is subterminal region (st) whereas, for the small chromosomes it is median (m) and sub-median (sm) regions.

Table 4. Summary of chromosome number counts of the studied species

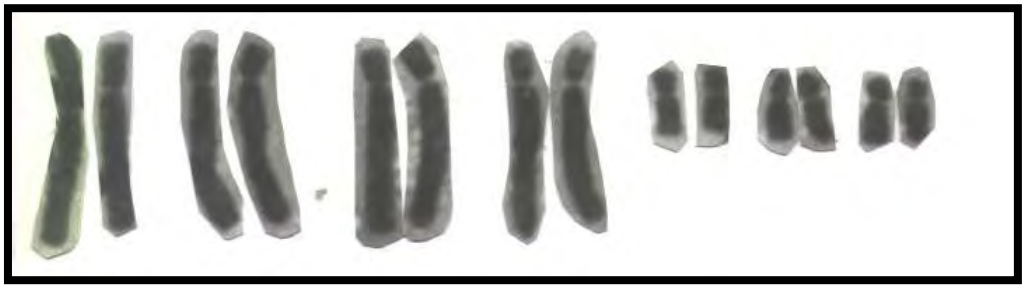
Species	Number of potted plants analyzed	Number of metaphase plates analyzed for each plant	*2n* Chromosome number
A. kefaenisis	1	5	14
A. harlana	1	6	14
A. trigonantha	1	4	14
A. yavellana	1	6	14
A. debrana	1	4	14
A. schelpei	4	6-7	14 , 14 + 1
Aloe species from Bale	1	3	14

4.1. *Aloe* species from Bale

This new species is diploid with the basic chromosome numbers of (x = 7) and 2n = 14 (Fig 2-A).The length of the four long chromosomes ranges from 15.30 µm to17.43 µm and the three short chromosomes have a narrow range of lengths of 5.26 to 5.92 µm (Table 5). According to the arm ratio, which was determined by pixel measurement, the four pairs of large chromosomes are st type, one of the short chromosome pairs is m type and two pairs are sm type (appendix 1).The karyogram of the species is presented in Fig 2-B.



A

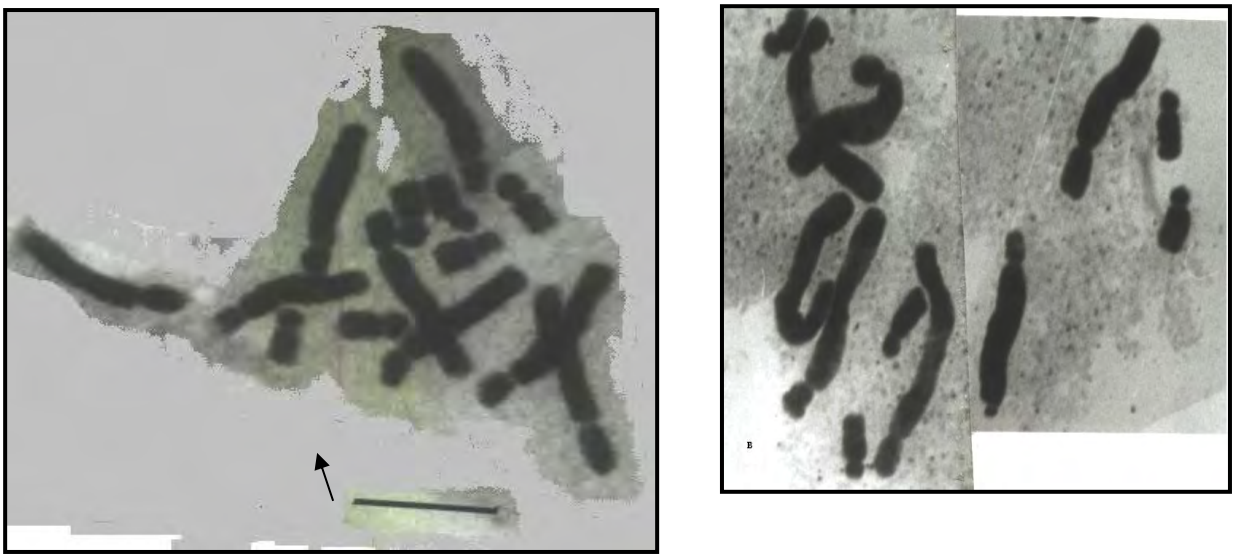


B

Figure 2- Chromosomes of an *Aloe* species from Bale. A. somatic metaphase chromosomes (2n = 14); B. The karyogram prepared from chromosomes shown in A. Bar = 10µm.

4. 2. *A. yavellana*

The present study showed that *A. yavellana* also has 2n = 14 chromosomes (Fig. 3-A) with basic number of 7. The lengthes of the large chromosomes range from 11.68 µm to 13.82 and that of the three pairs of small chromosomes is from 4.44 to 5.26 µm (Table 5). A satellite was also observed at the tip of the long arm of one of the large chromosomes at early stage of metaphase, indicating that at least one pair of the satellites is on the large chromosomes. (Fig. 3-B). The pixel data indicated that the species has 4 st, 1 m and 2 sm type of chromosomes (appendix 2). The karyotype is bimodal like that of the other species (Fig 3-C).



A **B**

C

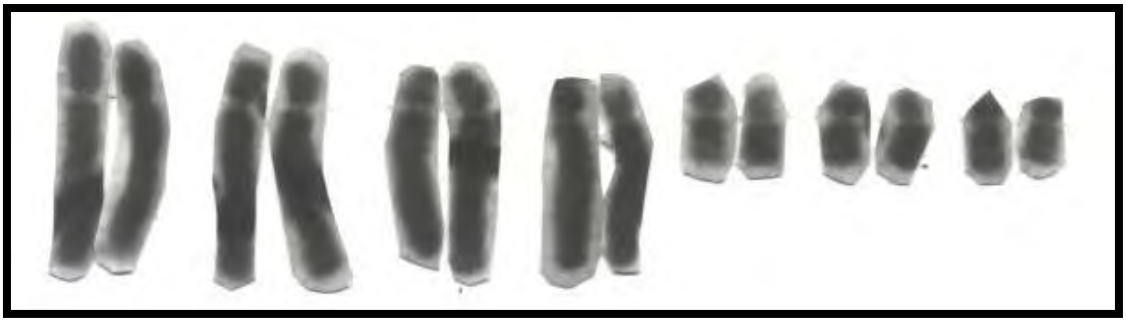
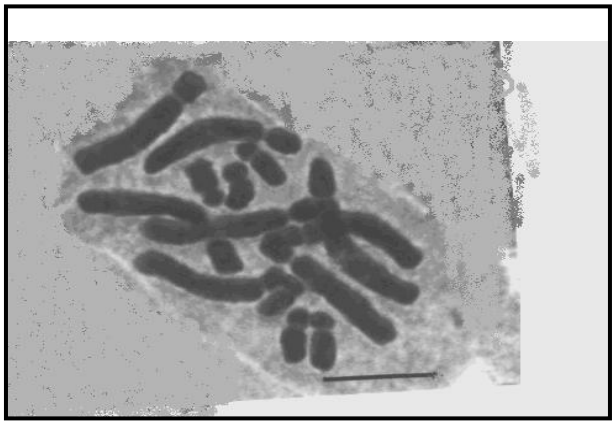


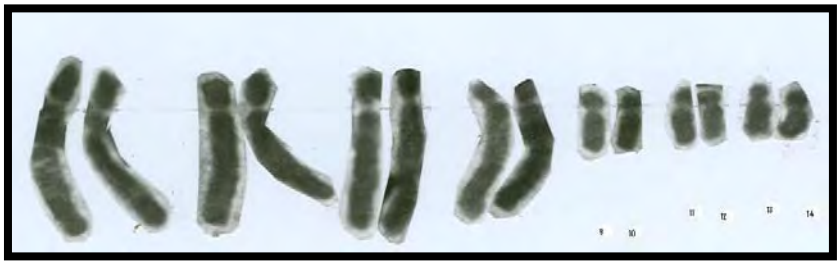
Figure 3- Chromosomes of *Aloe yavellana*. A. Metaphase plate with 14 chromosomes; B. partial complement with one chromosome showing a satellite at the tip of its long arm (arrow); C. the karyogram constructed from A. Bar = 10µm.

4.3. *A. trigonantha*

A 2n of 14 chromosomes was observed for this species as well (Fig 4-A). The large chromosomes have size range of 13.16 – 15.95 µm and the three small pairs have lengths of 5.1 to 5.26 µm (Table 5). According to their centromeric position, the four pairs of long chromosomes are st, and one pair of the short chromosome is m and the other two pairs are sm (appendices 3).



A



B

Figure 4- Chromosomes of *A. trigonantha.* A Metaphase plate with 14 chromosomes; B. Karyogram constructed from A. Bar = 10 µm.

4.4. *A. schelpei*

Of the four specimens of the species studied, three have x = 7, and 2n = 14. Exceptionally, at present study, one specimen of this species was observed to possess an additional small chromosome in addition to the normal complement of 14 chromosomes (2n = 14 + 1). As in other species, *A. schelpei* has a bimodal karyotype, consisting of 4 long chromosomes, which range in length from 14.14 – 16.62 µm, and three pairs of small chromosomes whose lengths range from 4.14 to 5.76 µm (Fig.5-A). The bimodal karyotype of *A. schelpei* is shown in Fig 4-B. One pair of the long chromosomes can be distinguished from the other chromosomes of the group by its relatively large size of the short arms and thus has relatively smaller arm ratio (r = 2.46). It is more of a sub-median (sm) type of chromosome whereas the remaining three pairs of chromosomes have r values 3.83 – 4.58, and so are st types (appendix 4). Thus, the karyotypic formula may be written 3 st + 3 sm +

1 m or 3 st + 3 sm + 1m + 1 s (s = supernumerary), the latter karyotypic formula being for the specimen with a supernumerary chromosome.

A

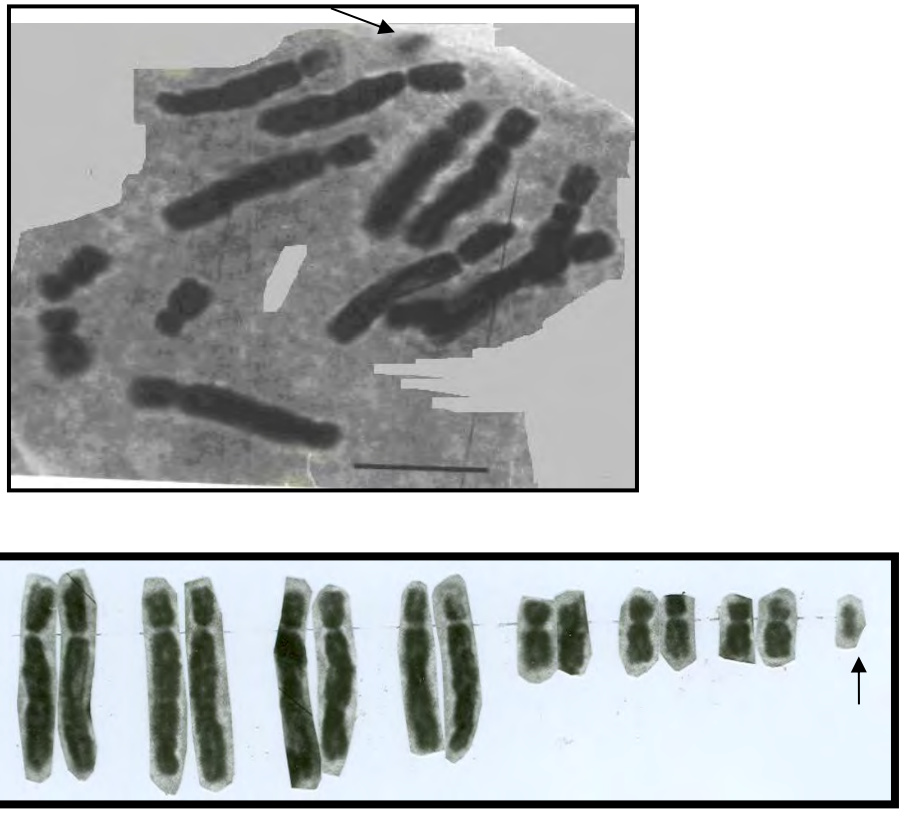
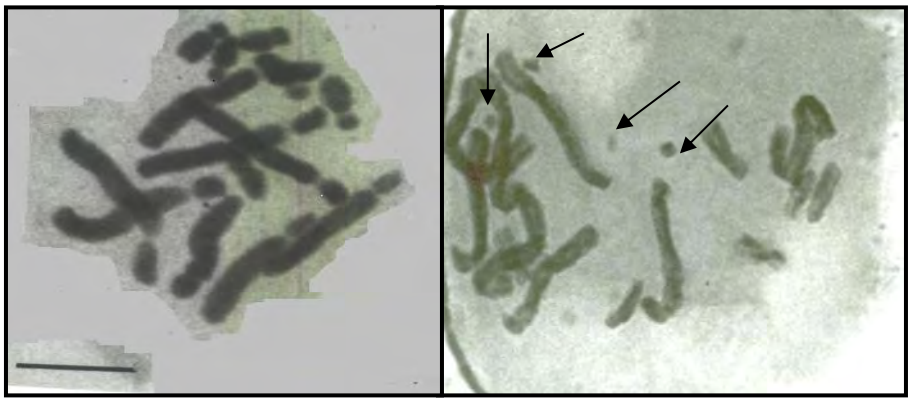


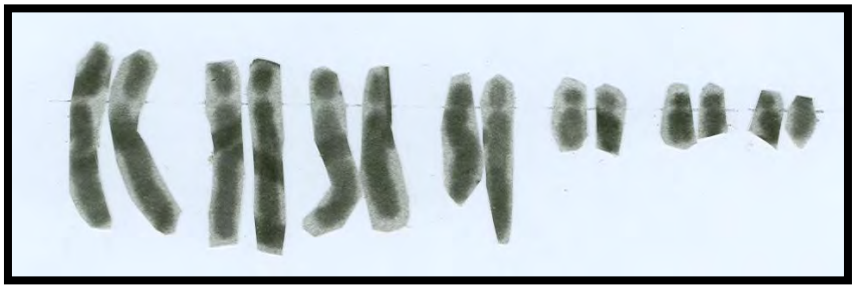
Figure 5- Somatic chromosomes of *A. schelpei*; A. Metaphase plate (2n=14+1, additional chromosome arrowed); B. Karyogram constructed from A. Bar = 10µm.

4.5. *A. harlana*

Like the other members of the genus, this species also has 7 pairs of diploid chromosome number (Fig.6-A). The length of the 4 pairs of long chromosomes; range from 13.49 µm to 16.61 µm and that of the three chromosome pairs range from 4.11 µm to 4.93 µm (Table 5). The pixel measurement shows that the species has 4st long, 1m and 2sm short pairs of chromosomes (appendices 5), which is a bimodal karyotype (Fig 6-C). In addition, although it has not been possible to determine to which chromosome and chromosome arm they belong, 4 satellites have been observed at late prophase stage (Fig 6-B).



A B

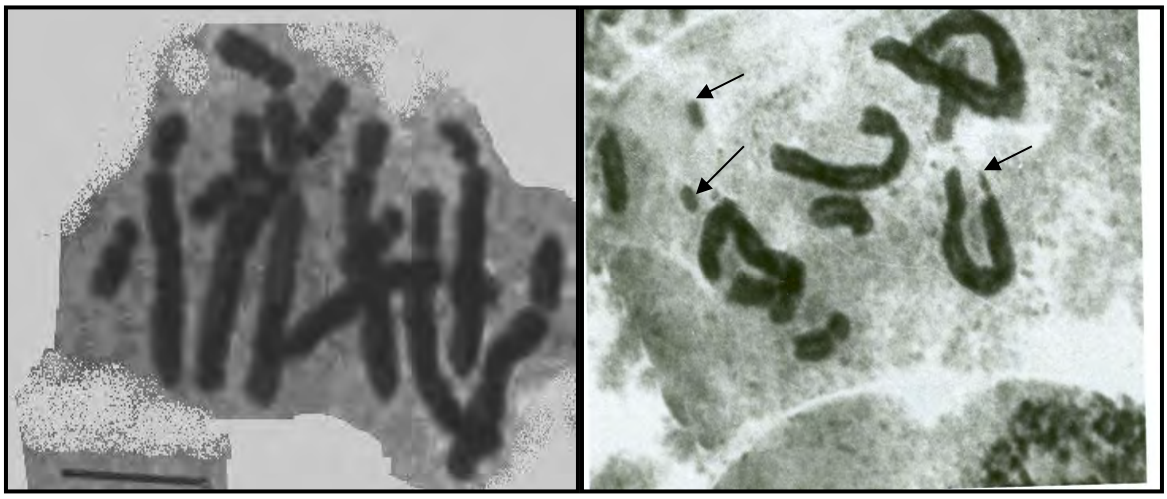


C

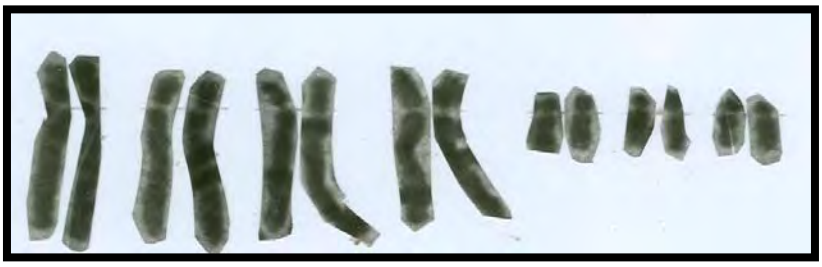
Figure 6- Somatic chromosomes of A. *harlana*, A. Metaphase plate with 14 chromosomes; B. Prophase cell showing 4 satellites (arrows); C. Karyogram prepared from chromosomes shown in A. Bar = 10µm.

4.6. A. *debrana*

Like the other species which were investigated in this study, this species also has 14 chromosomes, (Fig 7-A). The length of the 4 pairs of large chromosome is 15.46 to 18.26 µm, whereas the three pairs of small chromosomes have length of 4.93 – 5.43 µm (Table 5). Three satellites have been observed at an under condensed stage of the chromosomes; one of the satellites is located at the end of long arm of one of the large chromosomes, but it is not possible to assign the other two satellite to any one of the chromosomes. This observation indicates that the species has at least two pairs of satellite chromosomes, one pair being in one of the long chromosome pair, where as it has not been possible to assign the other pair of satellites to any of the chromosomes (Fig.7-B).While the 4 pairs of large chromosomes are st, one of the small chromosome pairs is m, the two are sm (appendix 6). The bimodal karyogram of *A. debrana* is shown in Fig.7-C.



A B



C

Fig- 7. Somatic chromosomes of *A. debrana*. A. Metaphase plate (2n=14); B. Part of prophase chromosomes with 3 satellites (arrows); C. Karyogram prepared from chromosomes shown in A. Bar = 10µm.

4.7. *A. kefaenisis*

In all the analyzed metaphase plates of A. *kefaenisis* the chromosome number of 2n = 14 was observed (Fig.8-A).The karyotype, like as in other species, consists of 4 pairs of long and 3 pairs of short chromosomes, with the length range of 14.66 µm to16 µm for the former and 5.33 µm to 6.00 µm for the latter groups of chromosomes (Table 5). In figure 8-C, a representative karyogram of A. *kefaenisis* is shown, however, due to the absence of good view of the last homologus pair of the smallest chromosome, it is not possible to display it here. The chromosome measurements, arm ratios, and position of centromers are

given in (appendix 7), which shows that like the other species, this species also has three types of centromeric position st, m and sm, which comprise 4 long pairs, one short pair and 2 short pairs respectively. During cytological analysis at late prophase stage, 2 satellites were observed (Fig 8 - B). One of the satellites (short arrow) appears to have been located on the short arm of a long chromosome. It is not possible to determine whether the second satellite is on the long arm of the same chromosome or it belongs to a different chromosome.

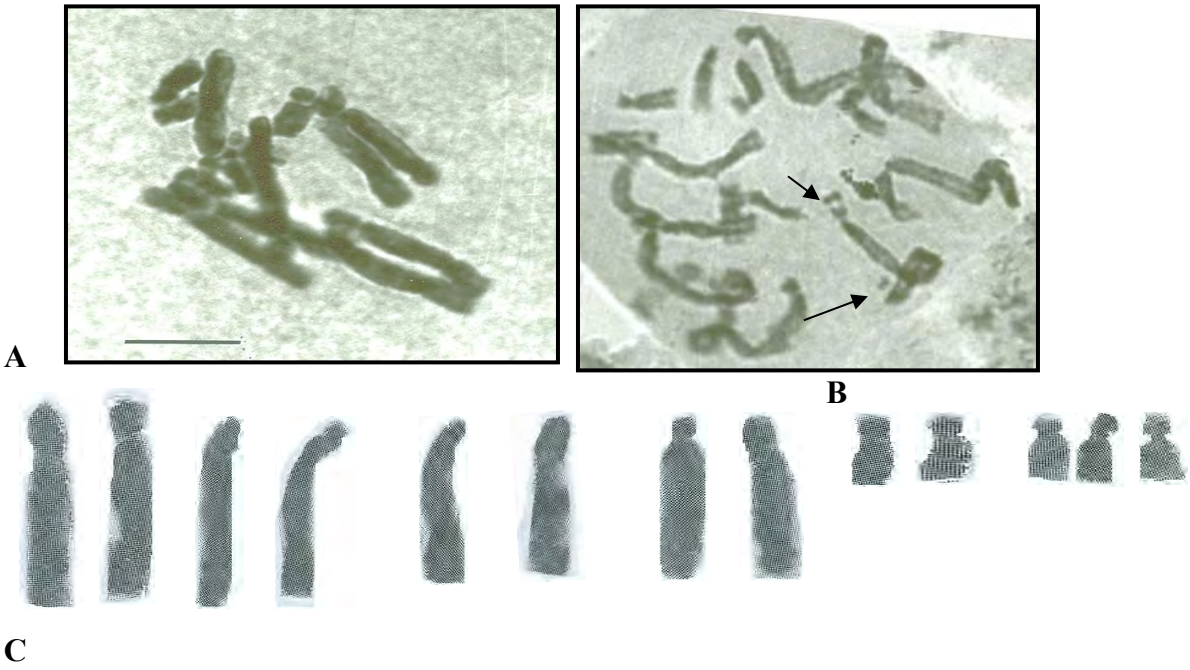


Figure 8. Somatic chromosome of *A. kefaenisis*. A. Metaphase plate (2n=14); B. 2 satellites (arrowed); C. Karyogram prepared from chromosomes shown in A. Bar = 10µm

Table 5. Chromosome size of the studied species

Species	Chromosome number and their size in μm						
	I	II	III	IV	V	VI	VII
Aloe from Bale (undescribed)	17.43	15.79	15.46	15.30	5.92	5.79	5.26
A. yavellana	13.82	13.49	12.5	11.68	5.26	4.77	4.44
A. trigonantha	15.95	14.8	14.14	13.16	5.26	5.1	5.1
A. schelpei	16.62	16.12	15.79	14.14	5.29	5.76	4.44
A. harlana	16.61	15.12	14.8	13.49	4.93	4.28	4.11
A. debrana	18.26	17.11	16.12	15.46	5.43	5.10	4.93
A. kefaenisis	16.00	15.33	14.66	14.66	6.00	5.33	5.33

5. Discussion

The endemic *Aloe* species examined in the present study are all diploids with the basic number x = 7, except that one of the specimens of A. *schelpei* an additional small chromosome was observed. This finding is in agreement with the works of Brandham (1971, 2004), Carter *et al* (1984), Brandham and Doherty (1998), and Mohammed Abate (2004). The present study has also confirmed the chromosome reports on *A. harlana* by Brandham (1971) and *A. debrana* by Fikre Dessalegn (1999).

Although chromosome sizes in *Aloe* vary from species to species with minor difference, they are karyotypically very similar in terms of relative size relationships between the chromosomes. The values obtained for species reported here were not significantly different from the values obtained for the other species studied previously (Brandham 1971, 2004), and Mohammed Abate (2004).

The position of the centromere, based on pixel measurement, indicated that all the endemic *Aloe* species examined have karyotypic formula of 4 st + 1 m + 2 sm, which is again in agreement with the work of Mohammed Abate (2004), except that in *A. schelpei* one pair of the large chromosomes appears more of sm type and one specimen harboured an accessory chromosome.

Owing to their small sizes, *Aloe* satellites are not usually detectable in condensed metaphase chromosomes and are rarely detectable on less condensed chromosome such as the prophase, or prometaphase stage chromosomes. In prophase stage, when the satellites are relatively better detectable, it is not usually possible to determine the chromosome on which they are borne, since the chromatin between the satellite and its chromosome is not

detectable (Brandham and Johnson, 1982; Aguilera and Buiza, 2003). Thus rDNA *in situ* hybridization is an option to determine the number and location of NORs and the location of the satellites (Adams *et al*., 2000).

The nature of the additional small chromosome observed in a specimen of A. *schelpei* is not known. It might be a B- chromosome or any other type of supernumerary chromosomes. It is obvious from the morphology of the chromosome that it is a metacenteric element. Since it is an element with a centromere, it is expected that this chromosome can behave normally during mitosis and is transmitted to the daughter cells through mitosis. It would also be interesting to observe how it behaves during meiosis. This could not be done in the present study as material did not flower in the greenhouse during the study period. Such chromosomes may originate as a centric fragement resulting from fusion of A – chromosome in Robertsonian translocation or from an amplification of the paracentromeric region of a fragmented A - chromosomes **(**Camacho *et al*., 2000). Whether this chromosome is an established B-chromosome that persists in the population or it is other type of supernumerary chromosome, which will eventually get lost from the population is not known. It would be interesting to carry out further study to establish whether it is B – chromosome or just a centric fragment floating around in the population until it gets eliminated by natural selection. If, up on future investigation, this chromosome turns out to be a B- chromosome, it would be the first to discover a B – chromosome in the genus *Aloe*.

Despite the fact that the present study has found bimodal karyotype, which is typical to the genus, there might exist minor structural differences between the karyotypes of different species, which might be revealed through meiotic analysis in inter-specific hybrids (Carter *et al*, 1984). Also, molecular cytogenetics such as GISH (Genomic *in situ* Hybridization) would help to detect transgressed chromosomes or chromosome segments between Aloe species that might result from interspecific hybridization (Stace, 2000).

The karyotypic evolution of the studied species clearly indicates stability as far as the chromosome number 2n=14 is concerned; this stability is shared by relative genera of

Gasteria and *Haworthia* of the Aloaceae family, in which the majority of species posses 14 acrocentric chromosomes (Brandham, 1971). Having two sharply distinct sizes of chromosomes, the *Aloe* karyotype can be considered as an evolutionarly advanced one, because a bimodal karyotype is considered asymmetric type of karyotype, which has undergone several type of breakage and reunion of chromosomal segments (Jackson, 1971). One possible evolutionary advantage of an asymmetric karyotype is that it might go through mitosis more rapidly than symmetric ones, because the smaller regions of acrocentric chromosomes or smaller metacentric chromosome could separate easily (Stebins, 1971).

From the present study there is some variation in the overall chromosome size from species to species, however, no major chromosomal differences were observed between the species; and thus in practice it is difficult to draw taxonomic conclusion simply from comparisons of chromosome complements of pairs of species, a phenomenon also encountered by many investigators (Brandham, 1971; Carter *et al*, 1984; Brandham *et al* 1994; Mohammed Abate, 2004). The similarity among genomes of *Aloe* species goes beyond mere morphological similarity of the chromosomes. Carter *et al*, (1984) studied chromosome behaviors during meiosis and pollen fertility in interspecific hybrids, and found that the hybrids showed good chromosome pairing and high chiasma frequency in the bivalents which demonstrated close affinity between genomes of their parent species. Further evidence which supports this idea was obtained, when interspecific hybrids of *Aloe* from Somalia were studied, and it was found that they exhibit close relationship both genetically and cytologically (Carter *et al,* 1984).

Even in a natural hybrid between *A. scobinifolia* and *A. peckii* the bimodal chromosome karyotype has been conserved. In contrast to this, in *A. pirottae*, an exchange of a small chromosome segment occurred between the L1 chromosome and one of the short chromosomes, as a result of which the short chromosome became slightly shorter than its normal homologue and the short arm of the long chromosome correspondingly became longer than that of its normal homologue (Brandham and Johnson, 1982).

Endemic *Aloe* spp. from different geographical regions of Ethiopia were included in the present study. As the result shows, all of them are diploid which means that none of them has undergone chromosome doubling of the same species (autopolyploid) or chromosome doubling in a diploid hybrid (allopolyploid); Incidence of chromosome doubling in aloes has been observed in *A. dawei* Berger, *A. elgonica* Bullock , *A. nyeriensis* , and *A. jacksonii* which are found in East and the Horn of Africa ; but majority of the species in the region are diploid, the reason may be due to non coinciding of flowering time, or fertilization might occur but embryo may fail to develop, or the hybrid is sterile because the homoeologous chromosomes do not pair (Carter *et al*,1984).

6. Conclusions and recommendations

6.1. Conclusions

The present study determined and confirmed the chromosome number, karyotype and ploidy levels of seven endemic *Aloe* species of Ethiopia. It showed that the studied species, all have chromosome number of 2n = 14, and it also confirmed that the basic chromosome number is (x = 7). Besides, a supernumerary chromosome has been obtained in one of the species, *A. schelpei*.

The karyotype of the studied species is a bimodal type, having eight large chromosomes and six short chromosomes, in which the size varies from 11.68 –µm to18.26 µm, and 4.11 µm to 6.00 µm for the long and short group of chromosomes, respectively. The karyotypic formula is 4 st + 1 m + 2 sm with minor exception observed in *A. schelpei*.

6.2. Recommendations

Characterization of *Aloe* species based on chromosome number and karyotype is very difficult because of the constancy in chromosome number and morphology across the species of the genus. In order to have sound result on cytological study, further study should include the chromosome behavior during meiosis and pollen fertility in inter-specific hybrids.

Furthermore, more advanced molecular cytogenetic techniques such as FISH and GISH would give valuable information about the number and location of nucleolar organizer regions and satellites as well as genomic differentiation among species. Additional molecular techniques would also reveal the phylogenetic and evolutionary status of the species. Besides, genome size measurement is important in determining the evolutionary

relationship of the species. Moreover, in order to detect the possible presence of B-chromosome in *Aloe*, further investigation is required at population level, at least in *A. schelpei* and closely related species.

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Appendices

Chromosome	Total length	Long arm	Short arm	Arm Ratio (L/S)	position of centromere
1	18.68	14.80	3.88	3.81	st
2	16.93	13.39	3.54	3.79	st
3	16.84	12.87	3.98	3.24	st
4	16.66	13.41	3.25	4.13	st
5	16.55	13.60	2.95	4.61	st
6	16.23	13.11	3.12	4.20	st
7	16.01	12.75	3.27	3.90	st
8	15.58	12.45	3.13	3.98	st
9	6.30	3.87	2.43	1.59	m
10	6.06	3.80	2.26	1.68	m
11	6.02	4.07	1.95	2.09	sm
12	5.90	4.00	1.90	2.11	sm
13	5.79	3.94	1.85	2.13	sm
14	5.53	3.73	1.80	2.07	sm

Appendix 1. Chromosome measurements, arm ratios and position of centromere in *Aloe* species from Bale

Magnification: 2200

Image resolution: 78.74 pixels per cm

Chromosome	Total length	Long arm	Short arm	Arm Ratio (L/S)	position of centromere
1	15.77	12.56	3.21	3.91	st
2	14.81	11.80	3.01	3.93	st
3	14.35	11.46	2.89	3.97	st
4	14.25	10.73	3.52	3.05	st
5	13.60	10.98	2.63	4.18	st
6	12.79	10.38	2.42	4.29	st
7	12.46	10.17	2.29	4.45	st
8	12.00	9.81	2.19	4.47	st
9	5.32	3.34	1.98	1.69	m
10	5.33	3.34	1.99	1.68	m
11	5.18	3.50	1.69	2.07	sm
12	5.17	3.29	1.88	1.75	sm
13	5.06	3.25	1.81	1.80	sm
14	4.45	3.13	1.32	2.36	sm

Appendix 2. Chromosome measurments, arm ratios and position of centromere in *Aloe yavellana*

Magnification: 2200

Image resolution: 78.74 pixels per cm

Chromosome	Total length	Long arm	Short arm	Arm Ratio (L/S)	position of centromere
1	12.29	9.36	2.93	3.20	st
2	12.10	9.49	2.61	3.63	st
3	11.51	9.37	2.14	4.38	st
4	10.75	8.39	2.37	3.54	st
5	10.68	8.31	2.37	3.51	st
6	10.12	8.09	2.03	3.99	st
7	9.94	8.38	1.56	5.36	st
8	9.72	8.03	1.69	4.76	st
9	4.00	2.22	1.78	1.25	m
10	3.99	2.44	1.55	1.57	m
11	3.93	2.60	1.33	1.95	sm
12	3.47	2.48	0.99	2.51	sm
13	3.40	2.23	1.17	1.91	sm
14	3.24	2.31	0.93	2.50	sm

Appendix 3. Chromosome measurements, arm ratios and position of centromere in *Aloe trigonanta*

Magnification: 2200

Image resolution: 78.74 pixels per cm

Appendix 4. Chromosome measurements, arm ratios and position of centromere in *A. schelpei*

Chromosome	Total length	Long arm	Short arm	Arm Ratio (L/S)	position of centromere
1	11.59	8.13	3.46	2.34	sm
2	11.31	8.18	3.12	2.61	sm
3	11.29	8.92	2.37	3.77	st
4	11.26	8.17	3.09	2.65	st
5	11.05	8.57	2.49	3.44	st
6	10.02	7.76	2.26	3.44	st
7	9.83	7.56	2.26	3.34	st
8	9.55	7.84	1.71	4.58	st
9	4.19	2.60	1.59	1.64	m
10	4.05	2.37	1.68	1.41	m
11	4.05	2.65	1.40	1.89	sm
12	3.94	2.55	1.39	1.83	sm
13	3.53	2.20	1.24	1.77	sm
14	3.43	2.18	1.25	1.74	sm
15	2.09	1.16	0.93	1.24	m

Magnification: 2200

Image resolution: 78.74 pixels per cm

Appendix 5. Chromosome measurements, arm ratios and position of centromere in *A. harlana*

Magnification: 2200

Image resolution: 78.74 pixels per cm

Chromosome	Total length	Long arm	Short arm	Arm Ratio (L/S)	position of centromere
1	12.05	9.03	3.01	3.00	st
2	11.99	9.97	2.02	4.93	st
3	11.66	8.36	3.30	2.53	st
4	11.65	9.01	2.64	3.42	st
5	10.62	8.50	2.12	4.02	st
6	10.18	8.56	1.62	5.28	st
7	9.48	7.28	2.19	3.32	st
8	7.89	6.38	1.51	4.22	st
9	3.90	2.48	1.48	1.68	m
10	3.83	2.30	1.53	1.50	m
11	3.38	2.34	1.04	2.26	sm
12	3.31	2.09	0.92	2.27	sm
13	2.97	1.99	0.98	2.03	sm
14	2.72	1.85	0.87	2.13	sm

Appendix 6. Chromosome measurements, arm ratios and position of centromere in *A. debrana*

Magnification: 2200

Image resolution: 78.74 pixels per cm

Chromosome	Total length	Long arm	Short arm	Arm Ratio (L/S)	Position of centromere
1	13.14	10.09	3.05	3.30	st
2	12.52	9.80	2.72	3.60	st
3	11.85	9.69	2.16	4.48	st
4	11.80	8.96	2.84	3.16	st
5	11.71	9.31	2.39	3.89	st
6	11.25	8.69	2.56	3.39	st
7	11.17	8.94	2.23	4.01	st
8	10.99	8.56	2.43	3.52	st
9	4.18	2.55	1.63	1.57	m
10	4.41	2.71	1.70	1.60	m
11	3.81	2.60	1.21	2.14	sm
12	3.81	2.66	1.15	2.30	sm
13	3.62	2.48	1.14	2.18	sm
14	3.57	2.46	1.11	2.21	sm

Appendix 7. Chromosome measurements, arm ratios and position of centromere in *A. kefaenisis*

Magnification: 2200

Image resolution: 78.74 pixels per cm

Chromosome	Total length	Long arm	Short arm	Arm Ratio (L/S)	position of centromere
1	12.60	9.48	3.12	3.04	st
2	11.76	9.24	2.51	3.67	st
3	11.71	8.95	2.76	3.24	st
4	11.05	9.09	1.96	4.63	st
5	11.01	8.30	2.71	3.06	st
6	10.61	8.87	1.74	5.11	st
7	10.46	8.39	2.07	4.06	st
8	10.42	8.35	2.07	4.03	st
9	4.27	2.92	1.78	1.64	m
10	4.22	2.88	1.35	2.13	m
11	4.04	2.78	1.26	2.20	sm
12	3.56	2.36	0.86	2.74	sm
13	3.43	2.21	0.89	2.48	sm
14	3.35	1.96	0.85	2.31	sm