ADDIS ABABA UNIVERSITY

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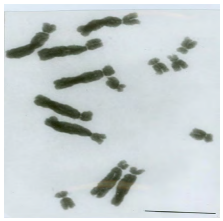
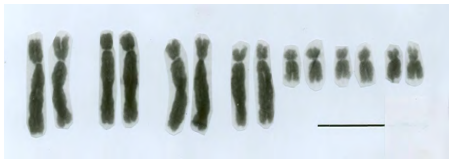
DEPARTMENT OF BIOLOGY

CHROMOSOME STUDY OF ELEVEN ENDEMIC *ALOE* SPECIES OF ETHIOPIA WITH EMPHASIS ON THE SATELLITES AND NUCLEOLI

BY

TEZERA TEMESGEN

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)

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Abstract

In the present work eleven endemic *Aloe* species of Ethiopia were cytologically studied. These species include: *A. friisii*, *A. otallensis*, *A. tewoldei*, *A. camperi*, *A. yavellana*, *A. harlana*, *A. schelpei*. *A*. *trigonantha*, *Aloe* species from Bale, *A. debrana* and *A. kefaensis*

Somatic chromosomes were prepared from the root tip meristem using colchcine solution or ice-cold pretreatment, followed by fixation (3:1, ethanol: acetic acid) and enzyme maceration in pectinase-cellulase solution. The air-dry slides so prepared were stained in Giemsa stain for chromosome morphological study or stained in silver nitrate to study the number of nucleoli. This is the first cytological study for *A. friisii*, *A*. *otallensis*, *A. tewoldei* and *A. camperi*. Also all the eleven species were studied for silver staining were for the first time.

The karyotype analysis has shown that all the species have 2n = 14 chromosomes, consisting of four pairs of large and three pairs of small chromosomes which form a bimodal karyotype. The maximum number of nucleoli observed varied among the species. Two nucleoli per nucleus was observed in *A. kefaensis*; four in *A. otallensis* and *A. debrana*; five in *A. tewoldei* and *A. friisii*; and six in *A. schelpei*, *A. trigonantha*, *A. yavellana*; *A*. *harlana* , *A. camperi* and *Aloe* species from Bale. The number of satellite chromosomes observed generally corresponded to the number of nucleoli. The satellites were observed on the long arm of long chromosomes and on the short arm of short chromosomes. These occurred in different combinations in different species. Thus, the species were observed to possess all the three pairs, or two long pairs, or one long pair, or one long plus one short pairs of chromosomes.

It was recommended to extend similar studies to the rest of the endemic species of *Aloe* and to carry out other studies which involve biochemical and molecular investigations in order to reveal more cytological and genetic variation which would help to elucidate the phylogenetic and evolutionary relationship among species of *Aloe*.

Key words: Endemic *Aloe*, Ethiopia, karyotype, satellite, nucleoli

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It has been suggested that the centre of origin for the genus is in the highlands of south East Africa, whence the ancestral aloes spread during the Tertiary Era (Sebsebe Demissew and Gilbert, 1997). The main centre of diversity for the genus is South Africa, with about 120 taxa. Other important centers of diversity are Eastern Africa and the island of Madagascar (Wabuyele *et al.*, 2006). In East Africa, there are nearly 200 taxa, many of which are naturally rare and confined to specific habitats (Sebsebe *et al.*, 2001). Many countries like Mallawi, Mozambique, Uganda, Zambia and Ethiopia have some endemic species. However, the highest rate of endemism is found in Madagascar and isolated Indian Ocean islands. Naturally, it is to be expected that some countries such as South Africa, and isolated islands, such as Madagascar, would have many endemic species (Newton, 2004).

1.2.1 Distribution and endemism of *Aloe* species in Ethiopia

In the area covered by the Flora of Ethiopia and Eritrea, 35 species (81%), out of the 40 *Aloe* species known so far are endemic (that is, have restricted distribution in one or two neighboring countries) (Sebsebe Demissew *et al*., 2001). The endemic taxa fall more or less into three main geographical areas in Ethiopia and Eritrea. A group of 14 endemics is restricted to the northern and central highlands, north and west of the Rift Valley. Another group includes 5 species which are restricted to the eastern highlands. The third group of 9 species belongs in the south. Quite a number of new species were described from Ethiopia in the 1990s and two additional new species have been discovered since the publication of volume 6 of the Flora of Ethiopia and Eritrea in 1997 (Sebsebe Demissew *et al*., 2001). The two species, so far not scientifically described, has been collected in Welega, unique within the genus in having traits making it resistant to fires (Sebsebe Demissew *et al*., 2001) and another species from Bale region, referred to as *Aloe* species from Bale, in the present study.

1.3 Habitat of *Aloe* species

The genus *Aloe* includes perennial plants that are adapted to highly disturbed areas and areas with extreme environmental conditions. This unique adaptation makes the aloes the most important groups of plants in such environments providing important resources of shelter, nectar food and moisture, especially to the avifauna (Oldfield *et al.*, 1998). In the wild, aloes occur in a wide range of soil types and substrates, including dolomite, granite, gypsum and limestone (Newton, 2004). They are also known to occur in a wide range of habitats at altitudes that vary from sea level to about 3300 m (Wabuyele *et al.*, 2006). Some species are arborescent, and others are virtually herbaceous, but all tend to be shallow-rooted and long-lived succulent plants (Holland, 1978).

The physiological adaptations in aloes, such as sunken stomata and a heavy wax cover on the leaf cuticle, make the plants particularly fitted for xeric habitats. The plants also possess traits that make them tolerant to disturbances, particularly the spiny leaves and bitter taste, which are deterrent to browsing (Wabuyele *et al*., 2006). *Aloe* species tend to be morphologically variable and geographically restricted and grow in areas with annual rainfall of between 200 and 800 mm (Holand, 1978).

1.4 Botany and Reproduction of *Aloe* species

1.4.1 Botany of the *Aloe* species

All *Aloe* species are perennial, leaf-succulent xerophytes (Newton, 2001). The plants are easily recognized by their rosettes of large, thick, succulent leaves, which are sometimes spotted. Although most are spiny succulents, aloes vary in size and morphology from dwarf rosettes ≈30 mm across to trees growing over 15 m high (Adams *et al.*, 2000). Primitive aloes have weak scandent stems, actinomorphic flowers and are almost mesophytic (e.g., *A. tenuior*). Slightly more advanced are *A. arborescens* and *A. acutissim* which have long stems but have zygomorphic flowers

and are more xerophytic. The advanced are the stemless extreme xerophytes (e.g., *A. peckii*) (Brandham and Carter, 1983)

Plants have developed various strategies, including chemicals and other mechanisms, to defend themselves against attack by herbivores, pathogens and insects (Horborne, 1997). Among them, chemical defense, by accumulating some secondary metabolites, such as phenol compounds may be the most important device (Fahey and Hans-Joachim, 1989). Aloe plants may have a chemical defense strategy to deter animals or insects from eating the leaves of these plants by accumulation of phenolic secondary metabolites (Hess, 1975).

1.4.2 Reproduction of *Aloe* species

Flowers of almost all *Aloe* species are diurnal, tubular, brightly colored red or yellow, unscented and produce abundant nectar. These features point to orinthophily as the pollination syndrome, and sunbirds are frequent visitors to aloe flowers in the field and in aloe gardens in Africa (Newton, 2004). The flowers are slightly protandrous, which means that the pollen grains mature and are released before the stigma become receptive and thereby, the flowers avoid self-pollination (Sebsebe Demissew and Gilbert, 1997). Consequently, hybridization is frequent in areas where two or more species of *Aloe* flower at the same time (Newton, 2004). Most *Aloe* species produce capsules, dry dehiscent fruits that split open at maturity to release the seeds. As the inflorescence sways in the wind, the seeds which are winged, are thrown out and blown away (Newton, 2004).

In addition to reproduction by qeeds, seferal species of *Aloe* have the ability to produce lateral rosettes that may develop into independent individuals by fragmentation. The new plantlets are capable of prkducing new roots and this is an important factor for maintaining the populations (Sebsebe Demissew and Gilbert, 1997).

1.5 Uses gf the *Aloe* species

1.5.1 Therapeutic use of the *Aloe* species

Aloe plants have long been the source of important products due to their nutritional and therapeutic values. *Aloe*, historically, is used as a laxative in a large dose or a stomachic in a small dose in the form of juice (or whole leaf) extract and as a healing agent of burns, skin ailments and wounds in the form of fresh juice for more than 20 centuries (Ki, 2006). Their leaves exudates are used to great extent in traditional medicines (Van Wyk *et al*., 1997).

The leaves of the genus *Aloe* are the source of two products that are quite different in their chemical composition and their therapeutic properties, aloe latex and aloe gel (Capasso *et al.*, 1998). *Aloe* latex is used for its laxative effects; aloe gel is used topically for skin ailment, such as wound healing, psoriasis, genital herpes and internally by oral administration in diabetic and hyperlipidaemic patients and to heal gastric ulcers (Mascola *et al*., 2004). There is also a preparation obtained from the whole leaf (aloe extract) which contains all the components present in aloe leaves. Aloe extract is potentially useful for cancer and AIDs (Mascola *et al.*, 2004).

1.5.2 Other uses of the *Aloe* plant

Aloe species have been cultivated for centuries for their ornamental, medicinal and cosmetic properties (Sebsebe Demissew *et al*., 2003) and health foods (Shioda *et al*., 2003). *A. vera*, *A. ferox* and *A. arborescens* are widely used as fresh food. *A. vera* and *A. arborescens* are widely used in Korea and Japan as ingredients of health foods and cosmestics. Some species of *Aloe* have been used in a wide range of skin and hair care products and have also formed the bases of health drinks and tonics (Park *et al*., 1998). Today, cosmetic industry manufacture and sell a considerable number of diverse products that contain aloe products as the ingredients of : lotions and creams of all kinds, shampoos and hair lotions, colorings, tooth pastes, deodorants, soaps,

shaving cream, sprays, bath salts, powders, lipstick, beauty masks, etc. (Schweizer, 2006).

Aloes leaves can be used as an insect repellent that is made by drying and burning aloes leaves and similar preparations are used to protect against ticks and stored food against weevils (Newton and Vaulgan, 1996).

1.6 Biological description, ecology and distribution of *Aloe* species used in the present study

In the present study, 11 *Aloe* species were used. These species are *A. camperi* Schweinf, *A. otallensis* Baker, *A. tewoldei* Gilbert and Sebsebe, *A. friisii* Sebsebe and Gilbert, *A. kefaensis* Gilbert and Sebsebe, *A. harlana* Reynolds, *A. debrana* Christina, *A. schelpei* Reynolds, *A. yavellana* Reynolds, *A. trigonantha* Leach, and *Aloe* species from Bale. A brief account of each species is presents below:

1.6.1 *Aloe camperi* Schweinf

Aloe camperi belongs to a group of caulescent aloes mainly characterized by erect, ascending or sprawling stems. It is distinguished from the other species occurring in northern Ethiopia by the cavate perianth which is 8-22 mm long and the small bracts 2-3 (-5) × 1-2 mm. The species grows abundantly on rocky slopes and sandy alluvial plains along the eastern escarpment; between 550 and 2700 m in Tigray and Welo floritic regions and in Eritrea. The main flowering period is from March to May (Sebsebe Demissew *et al*., 2003).

1.6.2 *Aloe otallensis* Baker

Aloe otallensis is unique among the Ethiopian aloes by the usually ornamented midribs of the outer perianth lobes, a character not known in any other species; the glaucous colour of the vegetative parts of the inflorescence; and the pale-pink

perianth and the large bracts (11-17 mm long) make the species very easy to recognize. The species grows in open *Acacia* bushland, often on dark soils between 1200 and 1600 m in Gamo Gofa and Sidamo floristic regions. The main flowering period is from September to December (Sebsebe Demissew *et al*., 2003).

1.6.3 *Aloe tewoldei* Gilbert and Sebsebe

The species belongs to a small group of aloes that occur in Ethiopia, Somalia and Kenya. The group is characterized by narrow, distinctly or obscurely spotted leaves which are separated along the erect or sprawling stems, and by the lax inflorescences. The species grows hanging from limestone cliff-faces, in Hararge and also in Bale floristic regions. The species flowered in cultivation in October (Gilbert and Sebsebe Demissew, 1996).

1.6.4 *Aloe friisii Sebsebe* and Gilbert

Aloe friisii belongs to a group of caulescent aloes mainly characterized by its paniculate inflorescence with yellow flowers. They have relatively small rosettes of narrow leaves and slender, sometimes trailing, stems and lax inflorescences of relatively broad flowers tapering slightly from a broad base. Leaves are narrowly elliptic and pale green with sparse whitish spots, these sometimes rather obscure. The species grows on rocky slopes in *Acacia horrida* bushland. The main flowering period is from January to February (Sebsebe Demissew and Gilbert, 2000).

1.6.5 *Aloe kefaensis* Gilbert and Sebsebe

Aloe kefaensis is widely cultivated as ornamental in Addis Ababa and Jimma. It is distinguished from other species of *Aloe* by the leaves being much less fleshy, the spots on the leaves being much more sparse or even absent, and the basal swelling of the perianth being less distinctly globose. The species grows in wooded grassland at around 1800 m in the Kefa floristic region. The main flowering period is from

September to November, occasionally also from February to June (Gilbert and Sebsebe Demissew, 1996).

1.6.6 *Aloe harlana* Reynolds

Aloe harlana is recognized by a brownish cartilaginous tissue along the leaf margins, usually forming a continuous edge between the spines, and by a bright yellow to red perianth. The species grows on sparsely vegetated slopes, often on limestone, between 1650 and 2400 m in Hararge floristic region. The main flowering period is in the rainy season from April to May, sometimes also from September to October (Sebsebe Demissew and Gilbert, 1997).

1.6.7 *Aloe debrana* Christian

Aloe debrana belongs to a group of aloes which often exhibit secondary branching and which usually are stemless, but some old plants develop thick, prostate stems. Leaves are in very dense rosette, spreading-recurved with dull green and old leaves drying brown. It is distinguished from the rest of the group by the small bracts 3-6.5 (-8.5) mm long. The species commonly grows in areas of grassland on thin soil overlying basalt, usually on gentle slopes between 2000 and 2700 m in Shewa, Gojam and Welo floristic regions. The main flowering period is in the dry season, from December to February (Gilbert and Sebsebe Demissew, 1996).

1.6.8 *Aloe schelpei* Reynolds

The species belongs to a group of caulescent aloes mainly characterized by erect, ascending or sprawling stems. *A. schelpei* is distinguish from the related species by the cylindrical perianth, 27-30 mm long, with the outer segments free for 12-15 mm, the (10-112-17 mm) long pedicles and the triangular-ovate bracts 6-8 × 2-4 mm. The species grows in more open areas of evergreen bushland on steep slopes and cliffs of basalt between 1700 and 2470 m in Shewa floristic region. The main flowering period is from October to March (Sebsebe Demissew and Gilbert, 1997).

1.6.9 *Aloe yavellana* Reynolds

The species belongs to a group of caulescent aloes mainly characterized by erect, ascending or sprawling stems. It is distinguish from the other *Aloe* species by their cylindrical-trigous perianth, 20-22 mm long, with the outer segments free for 8-10 mm long pedicles and the triangular-ovate bracts, 3-6 × 1-2 mm. The species grows in rocky slopes and in more disturbed areas near roads between 1600 and 1900 m in Sidamo floristic region. The main flowering periods is from September to October (Sebsebe Demissew and Gilbert, 1997).

1.6.10 *Aloe tringonantha* Leach

Aloe trigonantha belongs to a group of aloes that often has secondary branching and which is usually stemless, but some old plants may develop thick prostrate stems. It is distinguished from the rest of the group by the three angled perianth with truncate base. The species grows on dry stony ground near roads and along field margins between 1900 and 2100 m in Gonder and Gojam floristic regions. The flowering period is from September to January (Sebsebe Demissew and Gilbert, 1997).

1.6.11 *Aloe species* from Bale

A new endemic and yet undescribed *Aloe* species that has been added to the collection of the National Herbarium (ETH) is referred to as *Aloe* species from Bale. It is 40-70 cm tall, growing in clumps with 8-10 stems and it has 10-12 mm diameter. It has 1-2 inflorouscence per stem in which the leaves are spreading along the stem, the flowers secund and perianth is red. This species is found in Bale region, and grows at altitude between 1550-1660 m (Alemayehu Mekuria , 2007).

2. Karyological studies of chromosome

Cytological techniques determine the chromosome constitution of an organism and facilitate recognition of the individual chromosomes. Three terms, namely, karyotype, karyogram, and ideogram, are often referred to in the description of chromosomes (Singh, 2003). Karyotype is the sum total of chromosome number, size, shape, and length of haploid complement, ratio between the smallest and largest chromosomes (gradient index) and ratio between sum total of short and long arms (symmetry index) (Mandal and Gipson, 1998). Karyogram is the physical arrangement of the chromosomes from a photograph, where chromosomes are arranged in descending order (longest to shortest). An ideogram represents a diagrammatic sketch of the karyogram (Singh, 2003).

The classification of chromosomes is based on physical characteristics , such as size of chromosomes, features of telomere, position of centromere, secondary constriction, size and position of heterochromatic knobs, relative length of chromosomes, etc. (Singh, 2003)

2.1 karyotype

A group of plants or animals comprising a species is characterized by set of chromosomes, which have a certain constant features. These features include chromosome number, size and shape of individual chromosomes and other attributes (Gupta, 2004). The term karyotype is given to the group of characteristics that identifies a particular chromosome set and is usually represented by diagram called ideogram where chromosomes of haploid set of an organism are ordered in a series of decreasing size (Gupta, 2004).

Karyotype analysis has played an important role in the identification and designation of chromosomes in plant species (Singh, 2003). It also suggests primitive or advanced features of an organism (Gupta, 2004). The karyotypes of different groups are

sometimes compared and similarities in karyotypes are presumed to represent evolutionary relationships (Gupta, 2004). Species can be distinguished by a combination of karyotype formula and position of NORs in a particular chromosome pair. Karyotype length and asymmetry indexes are also useful to discriminate some taxa (Acosta *et al*., 2005).

A karyotype showing large differences between smallest and largest chromosomes of the set and having few metacentric chromosomes, is called asymmetric karyotype, which is considered to be relatively advanced feature when compared with symmetric karyotypes (Stebbibs, 1971; Gupta, 2004). A symmetrical karyotype is one in which the chromosomes are all of approximately the same size, and have median and submedian centromere (Stebbins, 1971). Asymmetry can occur either through the shift of centromere position from median to subterminal or terminal, or through the accumulation of differences in relative size between the chromosomes of the complement, thus making the karyotype more heterogenous (Stebbins, 1971).

2.1.1 Chromosome number

The analysis of chromosome numbers represents an important step in studies of genetic variation, phylogeny, taxonomy and evolution, as well as in studies on the structure and diversity of genomes (Jara-Seguel *et al*., 2006). To date, about 75% of flowering plants are not known cytologically, which suggests that many more are awaiting to be studied cytologically (Bennett, 1998). Chromosome numbers per cell range from 1 to over 600 pairs. A single chromosome is found in a species of ant and the nematode *Parascaris univalens* and at the other extreme, a fern has been described with over 630 pairs of chromosomes (Otto and Whitton, 2000). Until recently, the highest chromosome count is that of *Poa literosa* (in Family poaceae), 2n = 200. However, cytological work at Royal Botanic Gardens at Kew on *Voanioala geradii* (in Family Arecaceae) showed a count of 2n = 600 (Bennet, 1998) and the lowest chromosome number 2n = 4 in *Haplopappus gracilis* (in the Family Asteraceae) (Gupta, 2004).

2.1.2 Chromosome size

The most important morphological variable in chromosomes is the variation in size between the chromosomes of a genome. The chromosome in a genome can vary from being virtually all identical in size to exhibiting a size difference ratio of five or more (Stace, 2000). A chromosome is normally measured at mitotic metaphase and may be as short as 0.25 μm in fungi and birds, or as long as 30μm in some plants for example the genus *Trilium* (Gupta, 2004). According to John (1996), chromosomes may arbitrarily be classified as long (>10μm), medium (4-8 μm), or short (<2 μm)).

2.1.3 Chromosome Morphology

The position of the centromere is a useful landmark for the morphological identification and nomenclature of chromosome (Battaglia, 1955). In a particular chromosome, the position of centromere generally remains constant which provides a base for analysis of chromosome morphology by determining arms length and ratio (Mandal and Gipson, 1998). Levan *et al*. (1964) describe the nomenclature of chromosomes based on centromere position that determines the relative length of long and short arms. The relative length of the long arm (l) and short arm (s) are given by the arm ratio (r = l/s). Accordingly, centromeric position of a monocentric chromosome can be designated as shown below (Singh, 2003)

1, Median centromere is when the centromere is situated in the middle of the chromosome, resulting in arm ratio of about 1:1.

2, Submedian centromere is when the centromere is located near the middle of the chromosome, resulting in an arm ratio of more than 1:1, but less than 1:3 (i.e 1:1 to 1:2.9).

3, Subtelomeric centromere is when the centromere is near one extremity of the chromosomes, resulting in a ratio of 1:3 or more.

4, Terminal centromere when it is situated at one extremity of the chromosome, resulting in arm ratio of 1:0.

Levan *et al*. (1964) recommended the following nomenclature of centromeric position on the bases of arm ratio. Accordingly, chromosomes are classified as median (M) region when r is exactly 1, (m) (r = 1.00-1.69), sub-median (sm) (r = 1.7-2.99), subterminal arm (st) (r = 3.00 - 6.99) and Terminal (t) (r = 7.00 or more).

Chromosomes are also defined as small, medium or large according to their normalized lengths (percentage of the chromosome length of total haploid complement length): large chromosomes which have a value of greater or equal to 100 units (i.e. 10% or more of the total haploid complement) are grouped as large, whereas those having a value of below or equal to 80 units (8% or less of the total haploid complement) are classified a small chromosomes and chromosomes whose lengthes fall between 80 and 100 units are considered to be intermediate in size (Bogart, 1974).

2.2 Factors that cause karyotype evolution

2.2.1 Polyploidy

Polyploidy is a phenomenon in which chromosome complements are consisting of three or more basic sets. They arise from chromosome doubling in somatic cells, or through the functioning of cytologically unreduced gametes (De Wet, 1971). If the sets of chromosomes are belonging to same genome, it is referred to as autopolyploidy, whereas if they belong to different genomes it is allopolyploidy. The later is formed by interspecific hybridization followed by doubling of the chromosomes of the hybrid. Polyploidy often makes gene exchange possible between species that are isolated from each other at the diploid level. The genotype becomes buffered through polyploidy and foreign genomes can be absorbed, often with out severely affecting fertility because of autosyndetic chromosome pairing in the hybrids (De Wet, 1971).

Polyploidy is extremely common in all the major groups of plants except the gymnosperms, and so much attention has been paid to it (Lewis, 1980). At least 50% of the world's higher plants consist of polyploid taxa, and in grasses over 80% are polyploids (Greilhuber *et al*., 1988). In respect to growth habit, the higher percentages of polyploidy within the modern genera are found in perennial herbs, and the lowest in annuals (Gupta, 2004).

One of the biggest stumbling blocks to the successful establishment of polyploidy in sexual species is the requirement for genetically compatible mate. Perhaps because of this obstacle, successfull polyploidy establishment appears to be facilitating by selfing, asexual, and perennial plants (Bell, 1982; Ramsey and Schemeske, 1998). Triploidy has generally been thought to be an evolutionary dead-end, because triploids have very low fertility because they produce aneuploidy gametes, owing to problems of chromosomal pairing and segregation during meiosis (Otto and Whitton, 2000)

2.2.2 Causes of karyotype evolution

Karyotypes usually differ between organisms, even closely related ones, and many of these differences are due to chromosomal rearrangements. These include translocations and inversions, duplications and tandem fusions (Sumner, 2003). Robertsonian fusions and fissions are also special types of rearrangements, in which the centromeric regions of acrocentric or telocentric chromosomes fuse to form a single meta- or submetacentric chromosome (Sumner, 2003). All these types of rearrangements have occurred commonly in evolution. Chromosome rearrangements due to inverions, translocations, duplications, deletions, and Robertsonian fusions and fissions can amply explain the origin of the vast range of karyotypes (Stace, 2003). Differences in karyotypes have aided many taxonomic decisions and have provided telling clues in unraveling evolution, e.g., in tracing the percentage of hybrids or the origin of genomes in polyploids (Stace, 2000).

3 The Chromosomes of *Aloe* species

3.1 Chromosome number and size

The Family Aloaceae is one of the most stable angiosperm families as far as gross chromosome morphology and basic number are concerned (Brandham and Doherty, 1998). All the cytologically examined species of *Aloe* have the basic number x = 7 and a karyotype that is always bimodal (Brandam, 1971). All aloes have a basic genome structure of one long submetacentric (L1), three long acrocentric (L2, L3, L4), and three short acrocentric (S1, S2, S3) chromosomes with the short ones averaging 1.5-3 μm in length, and the long ones almost exactly 4.6 times longer in a sample of *Aloe* species (Brandham and Doherty, 1998; Adams *et al.,* 2000).

Although the basic karyotype of aloes is uniform throughout the family, major structural changes to the chromosomes are widespread, with Robertsonian fusions, large translocations and other types of interchanges reported to be frequent (Brandham and Doherty, 1998). There is considerable interspecific variation in overall chromosome size in aloes as reflected in the nuclear DNA C-value. The 1C DNA amount varies, e.g., in diploids from 10.5 pico gram (pg) in *A. tenuior* Haw (which is a primitive species) to 23.9 pg in *A. peckii* Bally and Verdoorn (2004), which is an evolutionarily advanced species (Brandham, 2004).This indicates that DNA amount increases with evolutionary advancement in *Aloe* species. The increased amount of DNA is distributed along the long and short chromosomes in proportion to their lengths, thus maintaining a uniform bimodality of the karyotype in the genus that is independent of the variation of the total genome size and DNA amount (Brandham and Doherty, 1998). Certainly, no rearrangements disrupting bimodality have become fixed during *Aloe* speciation, which indicates that strong selection pressure must be acting against such karyotpe changes (Adams *et al*., 2000).

3.2 Chromosome satellite

The mitotic stages, late prophase and prometaphase, and less frequently metaphase were suitable for the study of chromosome satellites (Kifle Dagne, 1994). The association of the satellite chromosomes with the nucleolus at late prophase makes this stage particularly suitable to show these interelationships. Stace (1980) indicated that, satellites are formed by secondary constriction on the nucleolar organizer chromosomes. Thus, the size of satellites can vary considerably depending on the relative position of the secondary constriction on a chromosome. Battaglia (1955) gives a terminology for satellites as follows: microsatellite has less diameter than the chromosome diameter and is small in size; macrosatellite has equal diameter with the chromosome diameter; and is large in size and linear satellite having the shape of a long chromosome segment.

The nuclear organizing or secondary constrictions of *Aloe* chromosomes have been known to vary in position on chromosomes in different species (Brandham, 2004). Brandham (1971) showed that *Aloe* plants have a considerable variability in number and position of satellites. These structures are usually observed on the long arms of chromosome one (L1), and in one or two of the other long chromosome pairs. Many species are heterozygous for satellite number or position (e.g, *A. scobinifolia*) and this might well be connected with hybridization following satellite interchanges (Brandham, 1998). With one exception, no particular combination of number or position of satellite was found to be restricted to any one genus in the tribe (Brandham, 1971). The exception is the existence of satellites on the short arms of a pair of short chromosomes, found in both varieties of *A. tenuior* and *A. scobinofolia*. Satellites were not found on the short arm of the long chromosome nor on the long arms of the short chromosomes.

3.3 Polyploidy in *Aloe* species

Polyploidy is uncommon in *Aloe* and is not uniform in its geographical distribution in the genus (Brandham, 2004). In Southern Africa, where large number of species occur, only *A. ciliaris* Haw. is known to be a polyploid, with a hexaploid (2n = 42). Polyploidy plays a small but significant role in the chromosomal evolution of the *Aloineae*. Within diploids, major structural changes occur (interchanges, inversion, etc), but do not become fixed as homozygote and are of no evolutionary significance in the tribe. The ancestor of the few polyploid aloes is better known, however, and morphological evidence (Jeppe, 1969) indicates strongly that diploid *A. tidmarshii* is the progenitor of the only known autohexaploid, *A. ciliaris*. This is the only known case of hexaploidy in the entire genus (Brandham, 2004).

Polyploidy is relatively more common among the East African aloes. For example, the Somali *A. inermis* Forssk. and *A. cremnophila* Reynolds, the Kenyan *A. juvenna* Brandham and Carter and the Ethiopian *A. jacksonii* Reynolds are tetraploids with 2n = 28 (Brandham and Carter, 1990). Mostly, they arise as the products of sporadic doubling of the chromosome numbers of unrelated diploid species that have stabilized through natural selection to produce tetraploid species. Cutler *et al*. (1980) found more substantial instance of evolutionary-significant of doubling chromosome number that is followed by diversification to produce a group of polyploid species. These are shrubby aloes of Kenya that gave rise to tetraploid group that comprises *A. dewei* Berger, *A. elgonica* Bullock, *A. nyerensis* Christina, *A. kedongenesis* Reynolds and *A. cheranganiensis* Carter and Brandham (Brandham and Johnson, 1982).

In self-incompatible aloes, an important step in the production of polyploidy is through the triploid formation which is, either directly obtained from the diploid by genetic non-reduction or via a tetraploid through chromosome doubling and backcrossing with the diploid (Brandham and Carter, 1990). The formation of triploids, e,g., in *A. jucunda* with 2n = 21 chromosome is a quite frequent local event

resulting from the fusion of normal gametes of diploid with a non-reduced one (Brandham *et al*., 2004).

3.4 Nucleolus, nucleolar organizer region and silver staining

3.4.1 Nucleolus

Nucleoli are found in most eukaryote nuclei, though their number and size vary from one kind of organism to another and from one stage to another in the development of a single organism (Stebbins, 1971). The shape of nucleoli can also differ markedly from one cell type to another, and even within a single cell (Hernandenz-Verdun, 2004). Since, as a rule, each haploid set of chromosomes includes a single chromosome with a "nucleolar organizer," the normal diploid nucleus contains two nucleoli (Frankhauser and Humphrey, 1943). The number of nucleoli may therefore be used as a criterion for the identification of polyploid individuals or races within a species and, with certain restrictions, for the determination of phylogenetic relationships between different plant species (Frankhauser and Humphrey, 1943).

The nucleolus is the site of ribosome biosynthesis, where the synthesis of ribosomal RNA (rRNA) and the assembly of ribosomes take place (Gupta, 2004). The nucleoli are formed at specific points on specific chromosomes, usually at secondary constrictions which either mark off a satellite or are located some distance from the end of chromosome (Frankhauser and Humphrey, 1943). During cell cycle, nucleoli are assembled at the end of mitosis (late telophase), remain active during interphase, and dissembled in prophase. Nucleoli develop from chromosomal sites, called nucleolar organizing regions (NORs) (Hernandenz-Verdun, 2004).

The number of nucleoli in most ineterphase nuclei can be lower than the number of NORs because of a marked tendency for nucleoli to fuse (Heitz, 1931; Kifle Dagne and Heneen, 1992). In some cells, all the nucleoli formed at telophase may remain

unfused, in which case the number of NORs, can be inferred from the maximum number of nucleoli observed among a population of interphase nuclei.

3.4.2 Nucleolus organizer regions (NORs)

Another important landmark used to distinguish chromosomes is the position of any secondary constrictions. These are the sites of origin of nucleoli, that is, the region of DNA that produces ribosomal-RNA (rRNA), and known as nucleolar organizing regions (Stace, 2000). The 5.8s, 18s and 28s RNA are coded by the rRNA genes in the NORs (Sumner, 2003). The localization of NOR sites is an important tool in certain studies, such as those in evolution and cytotaxonomy (Galetti, 1998). Further more, the mean number of NOR is used as a pathological marker for some malignant tumor (Pitch *et al*., 2000).

Frequently NORs are subterminal in position, so that they delimit a short piece of well stained chromosomes segment called satellite (Stace, 2000). The mitotic stage, late prophase and prometaphase, are suitable for the study of the satellite chromosomes (Kifle Dagne, 1994).

3.4.3 Silver staining

Silver staining is the cytogenetic method most commonly used to detect the position of NOR on chromosomes (Zurita *et al*., 1998). Silver preferentially reacts with acid proteins which bind the newly synthesized rRNA and only the NORs which were active during the preceding interphase are specifically detectable by silver staining (Goodpasture and Bloom, 1975; Howell, 1977). It is a highly selective method for staining interphase nucleoli and NORs on mitotic and meiotic chromosomes (Sumner, 2003). It is the characterstic of silver staining that, in species with multiple NORs, not all the NORs are usually stained. On mitotic chromosomes, the silver-staining material forms on the outside of the chromatids at the secondary constriction, and does not form part of the chromatin itself. No more than 10% of the nucleolar

proteins that stain with the silver during inerphase are retained on mitotic chromosomes, the rest dispersing into the cytoplasm (Sumner, 2003).

Silver staining clearly differentiates three elements: a strongly impregnated area, moderately impregnated peripheral zone and small chromatic bubble located in the strongly impregnated area (Sato, 1985). In interphase, silver staining is largerly confined to regions of the nucleolus known as the fibrillar centers (Sumner, 2003).

3.6 Chromosome staining

Staining chromosome with dyes allows recognizing different chromosomes within a cell based entirely upon features of size and shape (Stace, 2000). Staining is carried out with dyes that give strong colour with DNA, such as carmine, orcein or fuchsin, Giemsa (Feuligen), and these technique are ideal for ascertaining the number, size and shape of chromosomes, and to highlight the position of primary and secondary constrictions (Stace, 2000). Such conventional staining normally stains chromosomes uniformly along their lengths.

A lot of progresses in differential staining of chromosomes along their length have been made in the last half century, including C-banding, G-banding, Q-banding, etc. Heterochromatin has successfully been used to identify individual chromosomes, in a genome and any numerical and structural changes (Sumner, 2003).

4 Objectives of this study

4.1 General Objective

- To study chromosome cytology of some endemic *Aloe* species of Ethiopia.

4.2 Specific objectives

- To determine the chromosome number for four endemic species whose chromosome numbers have not been previously reported (*A. friisii, A. otallensis, A. camperi* and *A. tewoldei*).
- To describe the karyotypes of the above four species.
- To determine the number and position of satellites and nucleolus organizer regions of 11 endemic species including the above four.

4.3 Materials and methods

4.3.1 Plant Materials

The *Aloe* plants used in this study were collected from their natural habitats in different parts of the country, except for two species (*A. kefeanisis* and *A. yavellana*) which were obtained from the *Aloe,* Garden, Science Faculty, Addis Ababa University. Sample specimens of these species were transplanted in pots in the green-house at the Science Faculty of Addis Ababa University. Fresh root tips were used (for somatic chromosome study). The *Aloe* species used in this study are listed in Table 1.

Table1. Aloe species used in the present study and their sites of collection

No	Species name	Site of collection
1	*A. friisii*	Sawla (towards Wolyita Sodo)
2	*A. otallensis*	Arbaminch
3	*A. tewoldei*	Hissue village town (Bale)
4	*A. camperi*	Afar Iure (Eaikka)
5	*A. yavellana*	AAU, Science Faculty, A. garden
6	*A. harlana*	Harerge
7	*A. schelpei*	North Shewa
8	*A. trigonantha*	Gondar
8	*Aloe* species from Bale	Bale
10	*A. debrana*	North Shewa
11	*A. kefaensis*	AAU, Science Faculty, A. garden

A

B

C

D

E

F

G

H

I

J

K

Figure 1. Pictures of *Aloe* species used in the present study: *A. friisii*; B. *A. otallensis*; C. *A. tewoldei*; D. *A. camperi*; E. *A. yavellana*; F. *A.harlana*; G. *A. schelpei*; H. *A. trigonantha*; I. *Aloe* species from Bale; J. *A. debrana* and K. *A. kefaensis*.

4.3.2 Pretreatment of roots for chromosome preparation

For somatic chromosome studies, actively growing root tips from the potted plants were harvested separately from individual specimens growing in the greenhouse. Then, the root tips were either cold treated, by keeping them in ice water for 24 to 26 hours, or treated with 0.05 % colchicines or (8-hydroxyquinoline, 0.002 M) for 2 to 4 hours at room temperature. These treatments arrest the cell cycle at metaphase stage and as a result increase the number of cells at this sage. The root tips were then transferred to a clean vial containing fixative (ethanol:acetic acid, 3:1) and fixed for 1-12 hrs at 4^0C. After brief rinsing in distilled water, enzyme maceration was performed in 4% cellulase plus 4% pectinase solution in a water bath for about 1 hour at 35-37^0C, by which time the lower meristematic tip of the root detaches from the root up on little agitation as the result of enzyme digestion (Kifle & Heneen, 1992). After decanting the enzyme solution, the root tips were rinsed in several changes of distilled water (Kifle Dagne, 1994).

4.3.3 Air-dry slide preparation

The detached meristematic tip of the root were pipetted onto a glass slide, the water was blotted off using a filter paper (by touching the edge of the water drop) and root tips were mashed in a drop of fresh fixative and the cells were spread by strongly blowing on the slide. The slides were then allowed to air dry at room temperature (Kifle & Heneen, 1992). The air-dry slides were stained in Giemsa stain in Sorenson's phosphate buffer (pH 6.8) for about half hour followed by rinsing in the same buffer, air drying and mounting in DPX.

4.3.4 Silver staining

Some drops of aqueous silver nitrate solution were placed on air-dry slide preparations and covered with a piece of nylon cloth with the shape of a cover slip. The silver nitrate solution was prepared in the preparation of 1 g $AgNo_3$, 1 ml distilled water and 10 ul formalin (37% formaldehyde), (Heslop-Harrison and

Schwarzacher, 2000). The slides were incubated in a moist chamber (improvised by lining a large petridish with filter papers moistened with distilled water) at 60^0C until the nylon cloth turns dark brown after one hour or more. By removing the nylon cloth, the slides were rinsed in several changes of distilled water, air dried and mounted in DPX. A large petridish lined with moist filter paper was used as a moist chamber.

5. RESULTS

In the present study, eleven endemic *Aloe* species of Ethiopia were investigated cytological. The main emphasis of the study was on the karyotypes, number of satellite chromosomes, and nucleoli number. The number of satellites and interphase nucleoli were studied in all the eleven species whereas karyotypes were constructed for four species for whose karyotypes are not available. This is the first chromosome study for *A. friisii*, *A. otalensis*. *A. tewoldei* and *A. camperi*. The chromosomes of the rest of the species were studied before (Alemayehu Mekuria, 2007). However, there is no systematic study of the satellites and no information at all available on the nucleoli number of any of the eleven species investigated by the present study. The findings of the present study are presented in the following sections.

5.1 *Aloe friisii*

This is the first report on the chromosomes of this species. Like all the other species of the genus *Aloe* so far investigated, this species has 2n = 14 chromosomes, which consist of four pairs of large chromosomes, designated as L1, L2, L3, and L4, and three pairs of small chromosomes, designated as S1, S2, S3 (Fig. 2. B). There is a distinct size difference between the former and the latter groups of chromosomes, thus forming a bimodal karyotype. The size differences within each group of chromosomes (large vs small group) is small and within a group the chromosome varies in a gradual manner, whereas the size difference between the two groups is large and sharp. Within the large group the absolute chromosome size ranges from about 20.00 to 16.66 µm, whereas within the group of small chromosomes the range is between 6.00 to 7.33 micrometers.

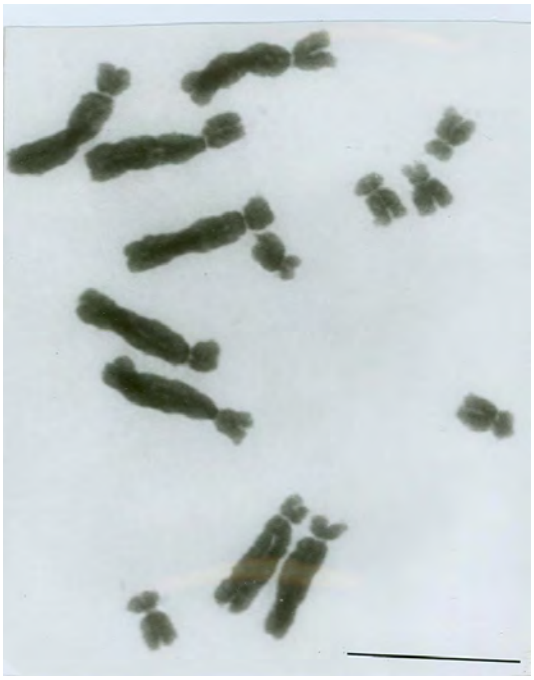
The four pairs of large chromosomes constitute about 79% of the total chromosome length of the complement, whereas the three pairs of small chromosomes make only about 21% of the total chromosome length of the complement of this species. On the basis of the arm ratio, the centromeric position of longest chromosome (L1) is of the sub-median (sm) type and that of the other three pairs of large chromosomes is of the

sub-terminal (st) type. On the other hand, all the three pairs of the small chromosomes are of the sub-median (sm) type. Thus, the karyotypic formula of this species can be presented as 1m +7sm + 6st.

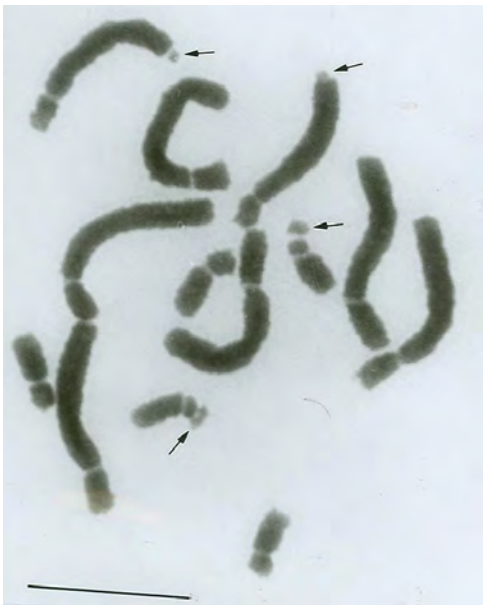
Satellites were observed at the end of the long arms of a pair of long chromosomes and on the short arms of a pair of short chromosomes (Fig.2C). In 98% of the cells examined, the number of nucleoli per nucleus ranged from 1 to 4. Only in four cases out of the total of 234 cells examined a nucleus was observed to contain more than four nucleoli (Table 2, Fig. 2D)

Table 2 Chromosome number, karyotypic formula, number of satellite chromosome and nucleoli.

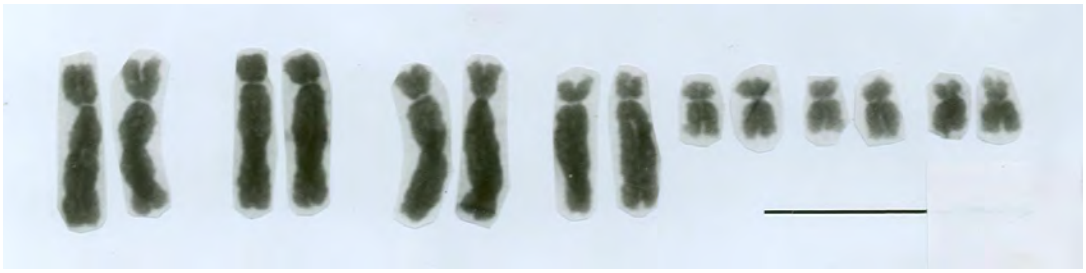
species	Chromosome number	Chromosome formula	Number of chromosome satellite
A. friisii	14	1m + 6sm + 7st	4
A. otallensis	14	4sm + 10st	4
A. tewoldei	14	1m +7sm + 6st	4
A. camperi	14	6sm + 8st	3
A. yavellana	14	1m + 6sm + 7st	4
A. harlana	14	7sm + 7st	5
A. schelpei	14	4m + 3sm + 7st	6



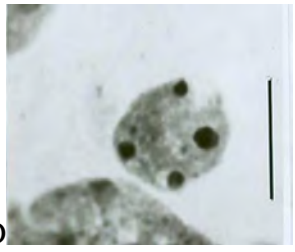
A



C



B



D

Fig.2. Chromosomes and nucleoli of *Aloe friisii*: A. Metaphase plate (2n = 14); B. Karyotype constructed from chromosomes in A; C. 13 moderately condensed metaphase chromosomes with four of the chromosomes showing satellites (arrows); D. Interphase nucleus with four nucleoli. Bar = 20 µm

5.2. *A. otallensis*

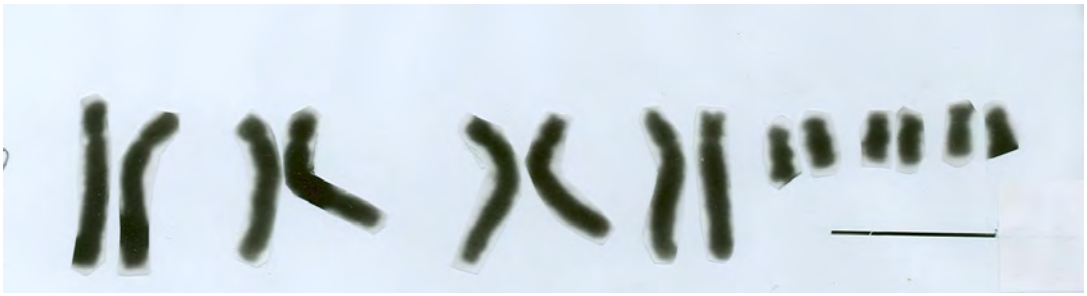
The present work is the first report on the chromosome cytology of this species. As in the preceding species, this species also shows the bimodal type of karyotype, with 2n = 14 chromosomes consisting of four pairs of large and three pairs of small chromosomes, with length range of 25.33 to 20.33 μm and 8.00 to 7.33 μm, respectively. The four pairs of large chromosomes constitute about 81% of the total 2n chromosome length of the species whereas the three pairs of small chromosome together contribute only about 19% to the total length of the chromosome complement. The arm ratio measurement shows that all the large chromosomes and two pairs of the small chromosomes are of the sub-terminal (st) type. One pair of the small chromosome has sub-median (sm) centromere. Thus, the karyotype formula is 4sm + 10st. The maximum number of nucleoli per nucleus observed for this species is four and minimum is one (Table3, Fig.3 D). As shown in Fig. 3 B, two pairs of the long chromosomes bear satellite at the end of the long arm. Size wise, the satellites on one pair of chromosomes are relatively larger than that on the other pair.

Table 3. Number and frequency of nucleoli in each cell nucleus of the species and their frequency in percentage in bracket.

	Number of nucleoli per nucleus and percent in bracket.						Total
species	1	2	3	4	5	6	
A. tewoldei	6 (3)	35(19)	72(39)	73(38)	1(1)	-	187
A. friisii	11(4)	72(31)	93(40)	54(23)	4(2)	-	234
A. otallensis	22(29)	21(28)	25(34)	7(9)	-	-	75
A.camperi	6(8)	39(52)	24(32)	5(6)	1(1)	1(1)	76
Aloe species from bale	2(2)	18(16)	42(38)	39(35)	9(8)	1(1)	111
A.yavellana	6(4)	23(14)	50(30)	48(29)	30(18)	9(5)	166
A.trigonantha	34(16)	61(28)	75(34)	43(19)	4(2)	3(1)	220
A.debrana	25(25)	35(34)	30(29)	12(12)	-	-	102
A.harlana	41(28)	47(32)	33(23)	19(13)	5(3)	1(1)	146
A.schelpei	22(9)	39(17)	56(24)	63(27)	35(15)	19(8)	234
A.kefaensis	31(44)	40(56)	-	-	-	-	71

A

B



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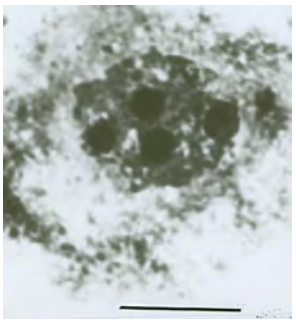
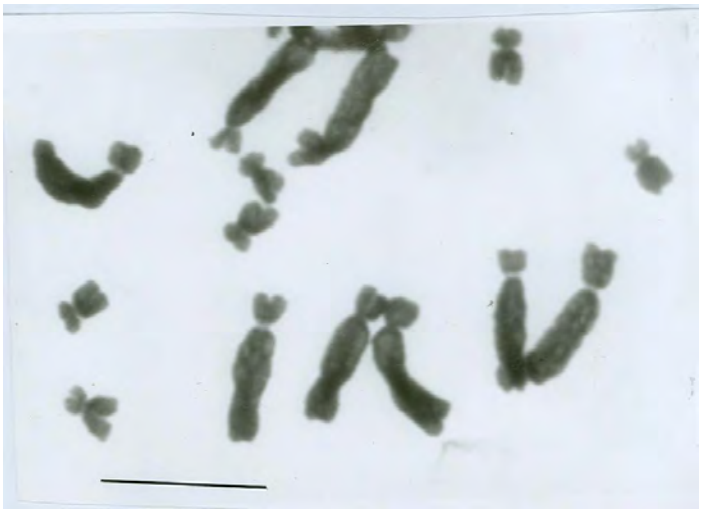


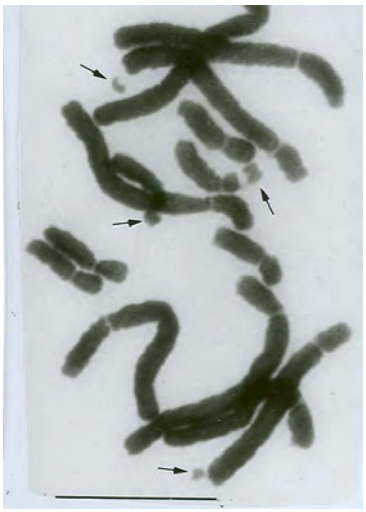
Fig.3. Chromosomes and nucleoli of *A. otallensis*: A. metaphase spread (2n = 14); B. Early metaphase stage chromosomes, showing satellites on the long arms of four long chromosomes; C. Karyogram constructed from chromosomes shown in A; D. Interphase nucleus with four nucleoli. Bar = 20 µm.

5.3. *Aloe tewoldei*

Like the other species of the genus *Aloe*, this species also has the bimodal type of karyotype with the four pairs of large and three pairs of small chromosomes. The former group of chromosomes has length ranging from 18.66 to 16.66 μm whereas the latter group has the range from 6.00 to 6.66μm (Table 4). The large chromosomes constitute about 78% of the total chromosome length. Of the large group of chromosomes, the first pair is sm type and the three remaining pairs are st type whereas all the members of the group of small chromosomes are sub-median (sm) type. Thus, the karyotypic formula is 1m + 7sm + 6st. In this species, 99% of the nuclei, had nucleoli number that ranged from 1 to 4 (Table - 3, Fig-4D). Four satellites were observed on the chromosomes of this species, one pair of which is at the end of the arm of a long chromosome pair and the second pair of satellite is on the short arm of a pair of short chromosomes (Figure 4B).

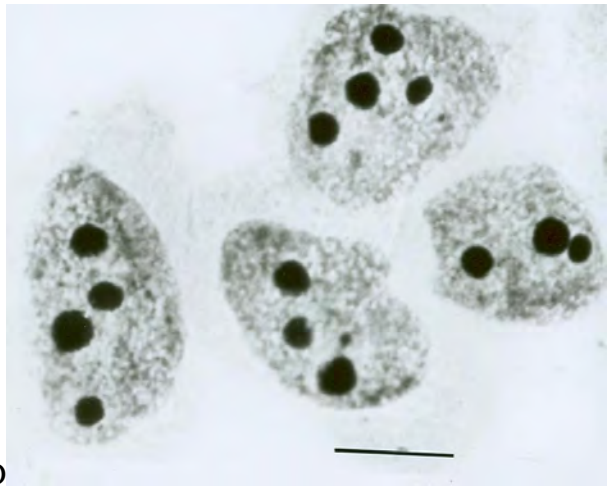


A



B

C

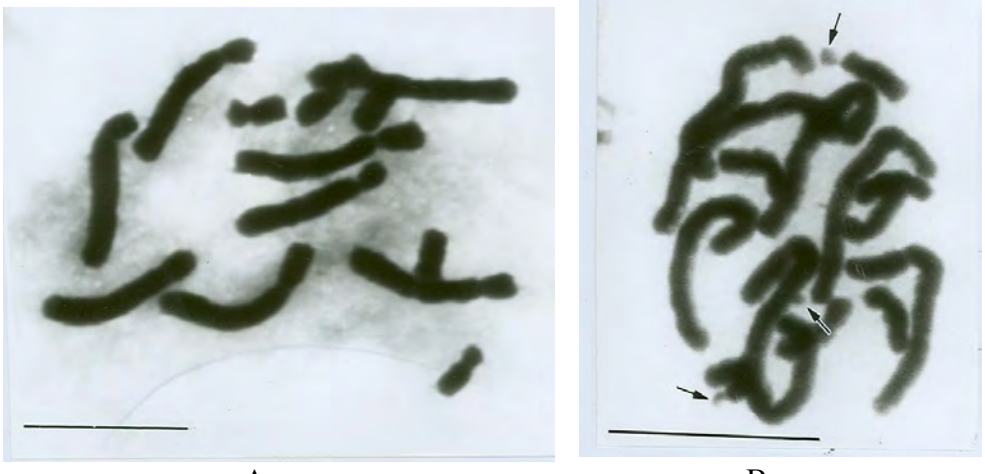


D

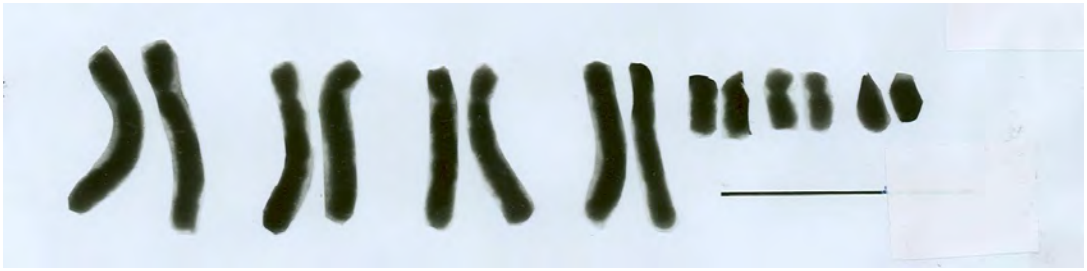
Fig. 4. Chromosomes and nucleoli of *A. tewolde*i: A. Metaphase chromosomes (2n = 14); B. Early metaphase chromosomes with satellites on the short arm of a pair of short and on the long arm of a pair of long chromosomes (arrows); C. Karyotype constructed from chromosomes shown in A; D. Four interphase nuclei, two with four and two with three nucleoli. Bar- 20µm

5.4. *Aloe camperi*

A. camperi has eight long and six short chromosomes (2n= 14). The long chromosomes of their length range of 26.6 to 19.66 µm and that of the short chromosomes range from 6.66 to 7.33 µm (Table-3). The two groups of chromosomes contribute 79 % and 21% to the total chromosome length, respectively. Based on the centric position, the largest pair is on the borderline between sm and st but it can be considered to be more of the sm type. The remaining three pairs of large chromosomes are clearly of the subterminal (st) type. All the three pairs of small chromosomes are sm type. Thus, the karyotyic formula is 6 sm + 8 st (Table-2). The number of nuclei containing 1 to 4 nucleoli per nucleus constituted 98 % of the total nuclei analysed (Table-2 & 3). Figure 5B shows a pair of long chromosomes and a single short chromosome bearing satellites. Assuming that the homologue of the latter is also bearing a satellite, there will be at least four satellite-bearing chromosomes in the complement of this species.



A B



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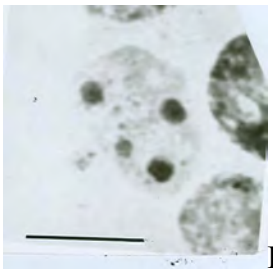


D

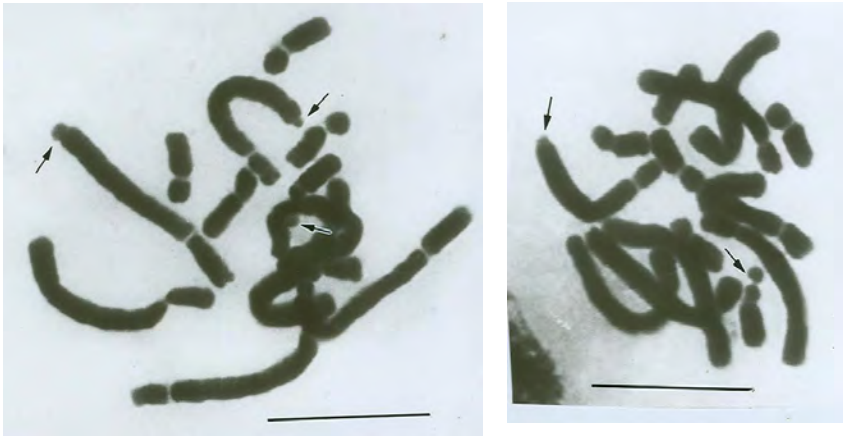
Fig.5. Chromosomes and nucleoli of *A. camperi*: A. Metaphase chromosomes (2n = 14); B. Late prophase chromosomes with satellites on the long arm of two long chromosomes, and on short arm of a short chromosome (arrows); C. A karyotype constructed from chromosomes in A; D. Interphase nucleus with four nucleoli. Bar = 20µm.

Table 4. Chromosome length of *A. friisii,A. otallensis ,A. tewoldei* , *A. camperi, A. yavellana*, and *A. harlana* in µm.

Chromosome length in µm							
species	L_1	L_2	L_3	L_4	S_1	S_2	S_3
A.friisii	20.00	19.33	18.66	16.66	7.33	6.66	6.00
A. otallensis	25.33	24.66	23.33	20.33	8	7.33	7.33
A. tewoldei	18.66	16.99	16.66	15.33	6.66	6	6
A. camperi	26.6	21.33	20.6	19.66	7.33	7.33	6.66
A.yavellana	15.5	14.6	14.3	13.65	6	5.6	5.15
A. harlana	23.65	21.65	20.6	19	8	6.6	6

5.5 *Aloe yavellana*

The chromosomes of this species are presented in Fig. 6. The karyotype is bimodal with four pairs of large and three pairs of small chromosomes, with chromosome length range of 16.00 to 13.3 and 6.00 to 5 .00 μm, respectively (Table-4). The large and the small chromosome groups contribute 78% and 22% to the total chromosome length of the complement, respectively. The karyotypic formula is 1m + 6sm + 7st. The maximum number of nucleoli observed in this species is six, and the frequency of cells with five and six nucleoli together constitute about 23% of the total of 166 nuclei examined (Table-3, Fig. 6D). The maximum number of satellites observed is three (Fig. 6 A & B), two of which are on the long arm of long chromosomes and one on the short arm of the short chromosome. From the size of the satellites and morphology of the chromosomes, it is apparent that the two long chromosomes belong to different homologous pairs, which suggests that there exist in the complement two pairs of long and one pair of short chromosomes bearing satellites. This corresponds to the maximum number of six nucleoli observed in fig 6 E.



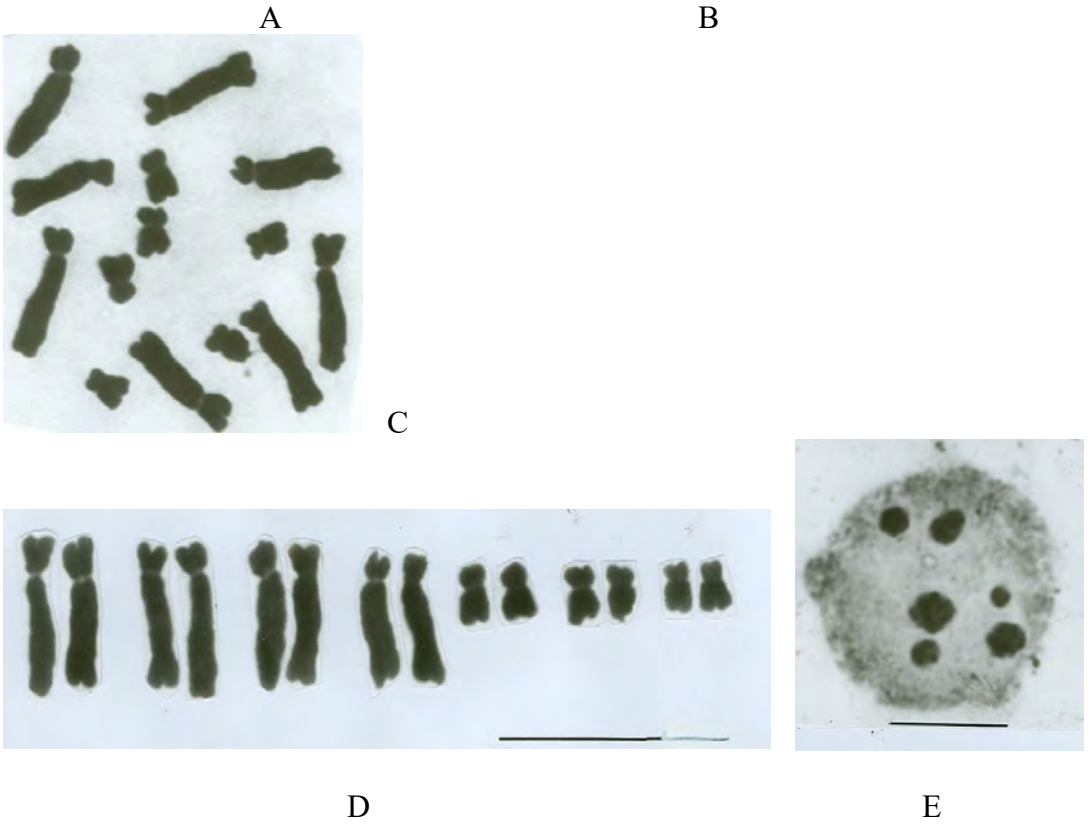


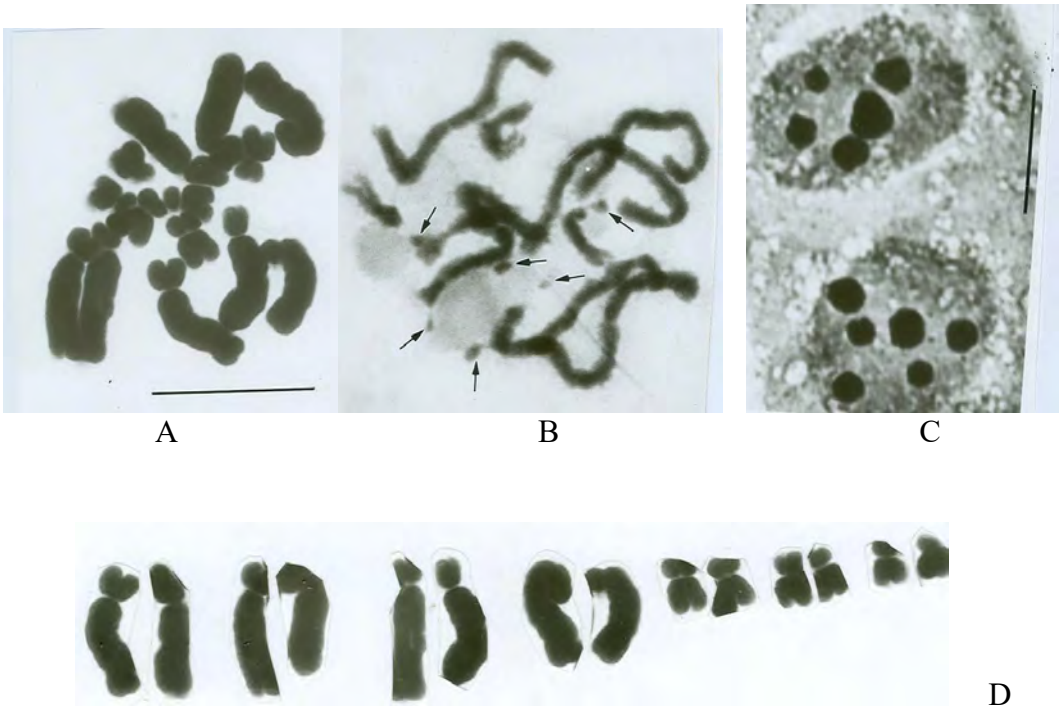
Fig. 6. Chromosomes and nucleoli of *A. yavellana*: A and B are cells with moderately condensed chromosomes showing satellites on the long and short chromosomes (arrows); C. Metaphase chromosome; D. karyotype constructed for chromosomes from chromosome C. E. Interphase nucleus with six nucleoli. Bar = 20µm.

5.6 *Aloe schelpei*

The chromosomes and nucleoli of this species are given in Fig. 7. The chromosome number is 2n=14 with the two class sizes of eight large and six small chromosomes, characteristic of the other species in the genus *Aloe.* the karyotype formula of this species is 4m + 3sm + 7st.

The maximum number of nucleoli at interphase stage per nucleus is six. Nuclei with five and six nucleoli together make about 23% of the total of the 234 nuclei

investigated for nucleoli number (Table 3). Corresponding to this, six satellites have been observed in a prophase stage (Fig. 7A). From Figure 7 A, it is possible to establish that pair of the satellites is connected to the short arms of a pair of short chromosomes. It can be assumed that the remaining four satellites belong to the long chromosome.

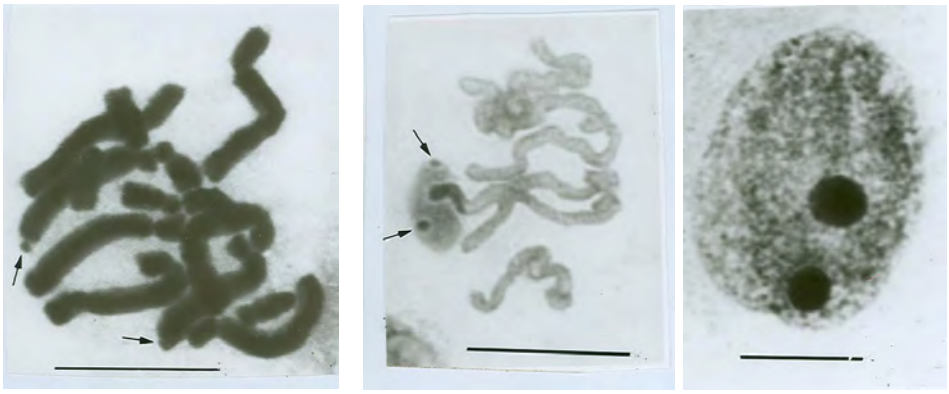


A B C D

Fig.7. Chromosomes and nucleoli of *A. schelpei*: A. Metaphase chromosomes (2n = 14); B. Prophase chromosomes with six satellites, five of which are associated with two nucleoli (arrows); C. Two interphase nuclei with five and six nucleoli. D. Karyotype constructed from A. Bar = 20 µm.

5.7 *Aloe kefaensis*

Aloe kefaensis too has 2n=14 chromosomes consisting of four large pairs and three small pairs (Fig 8 A). From among the *Aloe* species investigated in the present study, the lowest number of satellites and nucleoli were observed in this species. The maximum number of satellites observed is two which is the same as the maximum number of nucleoli observed per nucleus. About 44% and 56% of the nuclei contained one and two nucleoli, respectively (Table 3).

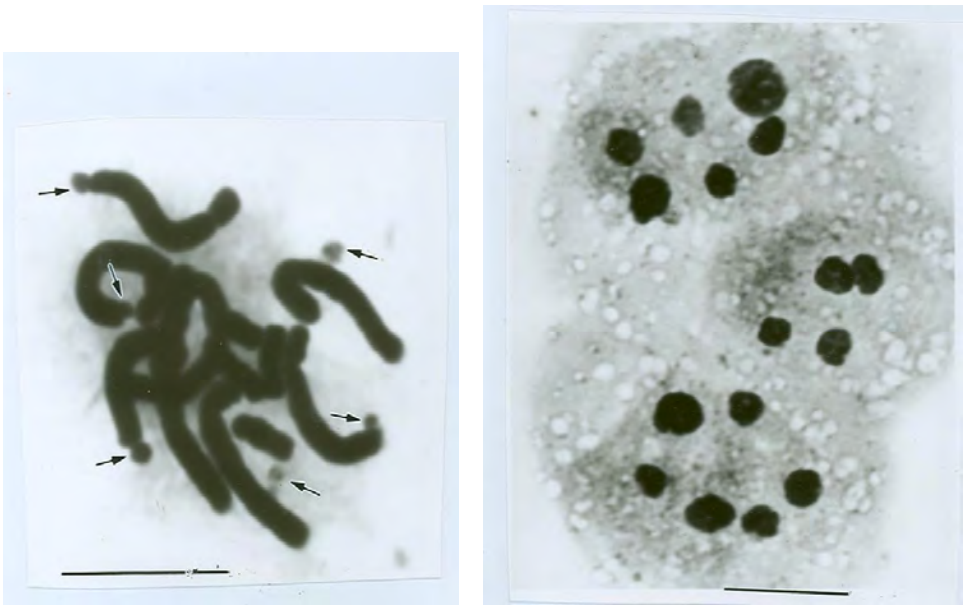


A B C

Fig. 8. Chromosomes and nucleoli of *A. kefaensis*: A. Metaphase chromosomes (2n = 14) with satellites on the long arm of two long chromosomes (arrows); B. Prophase stage showing two satellite chromosomes associated with a nucleolus with the satellites looking like dark dots (arrows); C. Interphase nucleus with two nucleoli. Bar = 20 μm.

5.8 *Aloe trigonantha*

Figures 9A & B present the chromosomes and nucleoli of *A. trigonantha* respectively. As could be seen from the figure, there are six satellites. Two pairs are located at the end of the long arms of two pairs of the long chromosomes. The size of one pair of the satellites is slightly larger than the other pair. Although the connection between the satellites and the chromosomes are not discernible from Fig. 9B, it can be assumed that the remaining pair of satellites belong to the short chromosomes. The six satellites correspond in number to the maximum number of six nucleoli per nucleus observed in about 3% of the nuclei (Tables 2 & 3).

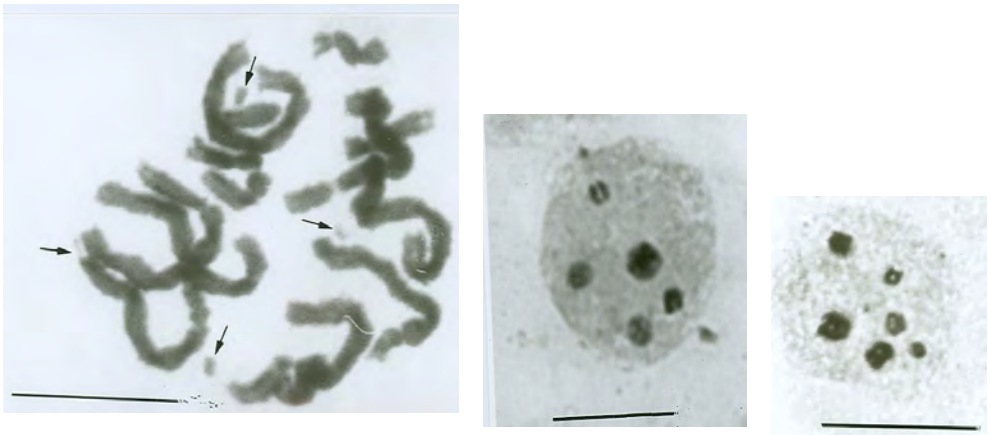


A B

Fig. 9. Chromosomes and nucleoli of *A. trigonantha*: A. Two long chromosomes pairs with satellites on the long arms and an additional pair of satellite belonging to small chromosomes (arrows); B. Three interphase nuclei, one with four and two with six nucleoli. Bar = 20 μm.

5.9 *Aloe* species from Bale

A late prophase chromosomes and a nucleus of this aloe are shown in Fig.10. The number of nucleoli in this species per nucleus ranges from one to five, with latter being observed in 8% of the cells, which implies the presence of six NORs, although the number of satellites observed were four, a pair on long chromosomes and a pair on short chromosomes, it can be assumed that there may be a pair of satellites that have not been detected (Figure 10A).

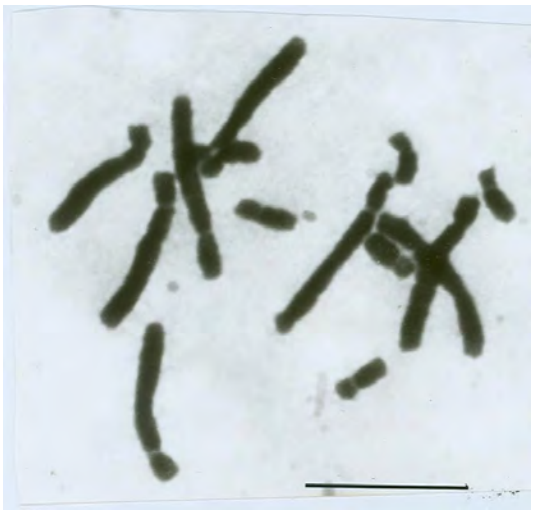


A B C

Fig.10. Chromosomes and nucleoli of *Aloe* species from Bale: A. Late prophase chromosomes with four satellites (arrows); B. B & C Interphase nucleus with five and six nucleoli respectively. Bar = 20 µm.

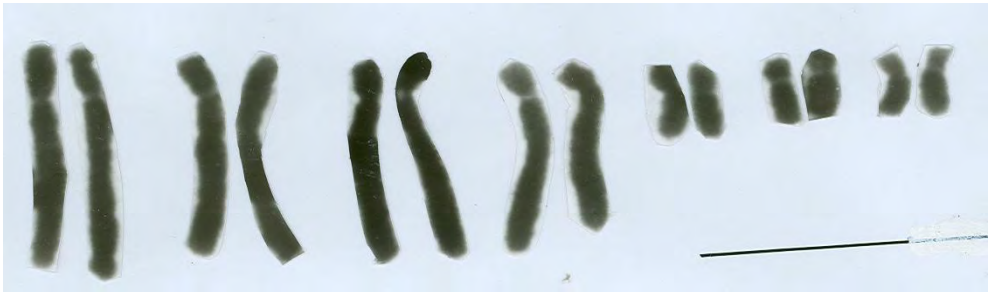
5.10 *Aloe harlana*

The chromosomes, satellites and nucleoli of this species are presented in Fig 11. The large chromosomes range in length from 23.3 to 18.00 µm, and the short chromosomes from 6.00 to 8 µm. The karyotypic formula is 7sm + 7st. The two groups constitute 80% and 20% of the total chromosome length of the complement, respectively. About 4% of the cells contain above four nucleoli (5 to 6 nucleoli) (Table 3). The maximum number of six nucleoli observed corresponds to the number of satellites observed (Fig.11 D). The two pairs of satellites of the long chromosomes show size difference.

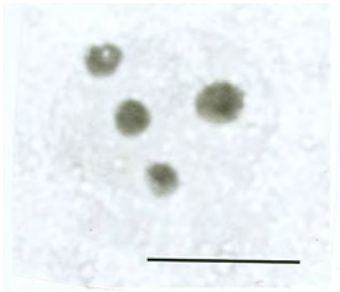


A

B



C

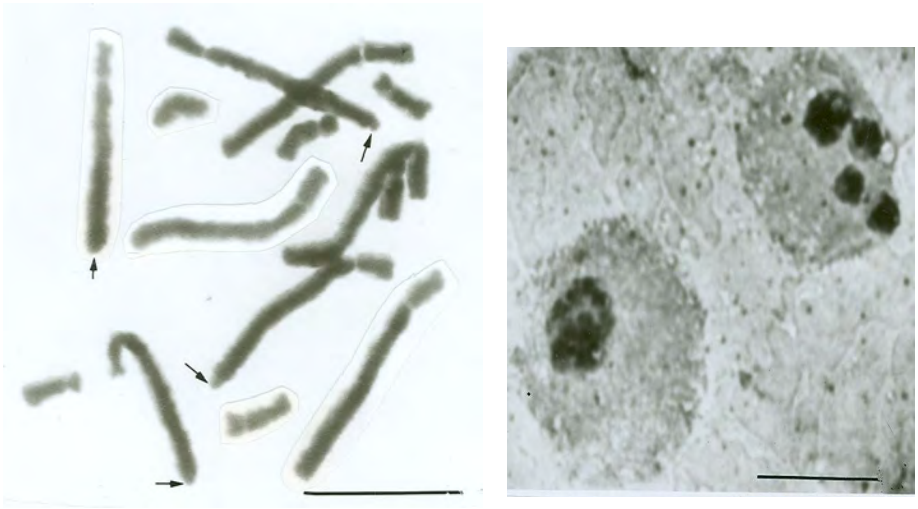


D

Fig.11. Chromosomes and nucleoli of *A. harlana*: A. Metaphase chromosomes (2n = 14); B. Early metaphase chromosomes with five satellites (arrows); C. karyotype constructed from chromosomes shown in A; D. Interphase nuclei with four nucleoli. Bar = 20 µm.

5.11 *Aloe debrana*

A. debrana bears four satellites and showed on long chromosome (Fig. 12B). The number of satellites, here also equal to the number of nucleoli. All the cells contained nucleoli number of four or less than four (Table 2).



A B

Fig.12. Chromosomes and nucleoli of *A. debrana*. A. Chromosome with satellites on the long arm of two pairs of long chromosome (arms). B. Two nuclei, one with a single large nucleolus and the other with four nucleoli. Bar = 20 μm.

6. DISCUSSION

The Family Aloaceae is one of the most stable angiosperm families as far as gross chromosome morphology and basic chromosome number is concerned (Brandham and Doherty, 1998). The diploid chromosome complement of all species so far investigated form a bimodal karyotype with eight long, submetacentric to acrocentric chromosomes and six short chromosomes (Brandham and Doherty, 1998). The karyotypic evaluation of the present study is in strong agreement with the reports of the above writers.

In the present study, variations in the length of chromosomes have been observed between species. For example, among the large chromosomes the largest was (26.6.00 µm) and was observed in *A. camperi* and the shortest (15.33 µm) in *A. tewoldei*. Similarily, among the small chromosomes, the longest (8.00 µm) was in *A. otallensis* and the shortest (5.00 µm) in *A. yavellana* (Table - 4). The length of chromosomes of *A. yavellana* and *A. harlana* which has been done by Alemayehu Mekuria (2007) has great differences with the present study on same species. However, it should be noted that the length of chromosomes depends upon the condensation stage of the chromosomes and thus variations may be observed even between chromosomes from different cells of the same individual unless they are at a similar stage of condensation. In view of this, it is difficult to determine how much of the size differences between chromosomes of different species is real and how much is due to differential chromosome condensation. Furthermore, to make a reliable comparison one has to have mean values of a number of measurements for each chromosome, which has not been possible to do in the present study.

Even though *Aloe* chromosomes show uniformity across the genus in the basic number and gross morphology, there may be cryptic structural differences such as deletions, inversions, and translocations (Brandham, 1971) that would not change the gross morphology of chromosomes. Conservatism in *Aloe* chromosomes is not only in its gross morphology, but also in the relative sizes of the large and small

chromosomes. In the present study, all the species for which chromosome measurements were made were found that the four pairs of long chromosomes constitute nearly 80% of the total chromosome length, whereas the small chromosomes contribute about 20%.

Silver-staining is a highly selective method to stain interphase nucleoli and the nucleolus organizer regions (NORs) on mitotic and meiotic chromosomes (Summer, 2003). In the present study, silver staining of the nucleoli was effectively achieved. However, no chromosomes with silver stained NORs were observed. This may possibly be due to the small size of NORs of *Aloe* chromosomes, or the technique used is not effective in staining the NORs. We found that the inclusion of formalin in silver nitrate solution gave a better result than without it.

According to Keller (2000) there is a direct relationship between the maximum number of nucleoli per cell and the total number of chromosomes in the complement with secondary constriction or NORs. However, the number of nucleoli varies from cell to cell and usually most of the cells of an organism contain less than the maximum number of nucleoli (Table 3). This could mainly be due to two reasons. Firstly, all the NORs may not be active in nucleolus formation in a given cell. Secondly, after being formed, nucleoli tend to fuse together as the cell progresses from telophase to interphase (Kifle Dagne, 1994). As the result of nucleolar fusion, the number of nucleoli in most of the interphase cells is usually less than the maximum number possible even if all the NORs in a cell are active (Kifle Dagne, 1994). Thus, the maximum number of nucleoli can be observed only if all the NORs in a cell are active and all nucleoli remain unfused. Such cells must be rare and thus a large number of cells have to be screened in order to find cells in which the maximum numbers of nucleoli are formed.

In the present study, variations in the maximum number of nucleoli have been observed between species. The maximum number observed for A. *kefaensis* was two nucleoli; the maximum numbers of nucleoli were observed in four species, *A.*

schelpei, *A. trigonantha*, *Aloe* species from Bale and *A. yavellana* (Table 3). The variation in the number of nucleoli within a species may be due to nucleolar fusion at the interphase stage. Interphase nucleolar fusion takes place during which NORs from more than one chromosome often participate in the formation of a given nucleolus (Hrnendez-Vardun, 2004). Hernendez-verdun (2004) also reveals that the shape, size and organization of the nucleolus vary with their activities. This is also seen in the present study. Howell (1982) described NORs were sites around which nucleoli reorganized during telophase-interphase. The number of secondary constriction in the chromosome complement agrees closely with the number of nucleoli because secondary constrictions contain NORs that are sites for the formation of nucleolus. Satellites are formed distal to the secondary constriction because of the under condensed chromatin in the secondary constriction. Therefore, the number of secondary constriction, number of nucleoli and number of satellites are closely correlated.

According to Brandham (1971) there is considerable variation in the number and positions of satellites in *Aloe* species. However, according to him, satellites are found neither on the short arms of long chromosome nor on the long arms of short chromosome. This is in agreement with our present finding.

With regards to the number of satellites, in the eleven *Aloe* species investigated in the present study, the maximum number of satellites observed was six. The presence of six satellites has been established for *A. schelpei*, *A. trigonantha*, and *A. harlana*, for which also a maximum of six nucleoli were observed. The lowest number of satellites, which is two, was observed in A. *kefaensis* (Table 2). This is also the species whose nuclei contain the lowest number of nucleoli per nucleus, i.e. two. Regarding *A. otallensis* and *A. debrana*, the maximum number of four nucleoli observed is corresponding to the number of satellites observed. Although four satellites were detected in *A. friisii*, *A. tewoldei*, *A. camperi* and the *Aloe* species from Bale, the maximum number of nucleoli observed was more than 4 (5 to 6) but occurred in a very low frequency (Table 3). This may suggest that there are more than four NORs

present in these species although we detected only four satellites. Thus, it may need further cytological investigation to look for an additional pair of chromosomes with satellite or application of more sensitive techniques such as fluorescence *in situ* hybridization (FISH) with ribosomal DNA probe (Fuku., *et al* 1994; Kifle Dagne *et al*, 2000) in order to determine the total NORs present.

In the present study, it has been observed that generally satellites are not detected on fully condensed chromosomes because, on condensed chromosome, the secondary constriction region (NOR) fully condenses like the rest part of the chromosome. So, one has to examine less condensed chromosomes of late prophase, premetaphase or early metaphase stage. Even in such cases, we have observed that, the number of chromosomes showing satellites vary from cell to cell of the same plant which suggest that some NORs do not involve in nucleoli formation or their secondary constriction condense earlier than that of others.

Stace (1980) indicated that satellites are formed by secondary constrictions on the nucleolar organizer chromosomes. There are also cases of satellite formation by non-nucleolar organizer secondary constrictions. In the present study the number of interphase nucleoli revealed by silver nitrate staining correspondeds to the number of satellite chromosomes observed, which indicates that all the satellites were formed by NOR secondary constriction and there is direct correspondence between the number of satellites and nucleoli. Mohammed Abate (2004) also showed the correlation between the maximum number of nucleoli and satellites in other *Aloe* species. His finding indicates that both the number of nucleoli and satellites range from 4 to 6. Furthermore, the correspondence between the number of nucleoli and the number of satellites observed shows the usefulness of silver nitrate staining of nucleoli as an indicator of the number of satellite chromosomes present in a species.

7. Conclusion and Recommendation

7.1. Conclusion

In the present study, the chromosomes of eleven *Aloe* species endemic to Ethiopia were studied. Karyotypes were constructed for seven of the species: *A. friisii*, *A. otallensis*, *A. tewoldei*, *A. camperi*, *A. harlana*, *A. yavellana* and *A. schelpei*. This is the first study of the chromosomes of the first four species. All the species showed diploid chromosomes number of 2n = 14, which consist of four pairs of long and three pairs of short chromosomes that form a bimodal karyotype. The number of nucleoli and satellites were analyzed in all the eleven species used in the present study. There is a direct correspondence between the number of satellites and nucleoli and both ranged from a minimum of two to a maximum of six. These satellites were observed at the end of the long arms of two pairs of long chromosomes and a pair of short chromosomes.

Variations were observed among species in the number and combinations of the three satellites chromosome. The combinations of satellited chromosomes observed in different species are: (a) two pairs of long plus one pair of short; (b) one pair of long plus one pair of short; (c) two pairs of long, and (d) one pair of long satellited chromosomes.

The result showed that in spite of the conservation of the gross morphology of *Aloe* karyotype, there are differences in the number and type of satellited chromosomes among *Aloe* species. From the studied species, the highest number of nucleoli and satellites observed are six. This indicates that, there is a close association between number of nucleoli and number of satellites. This helps to determine the number of NORs indirectly, because secondary constriction is a site of NORs and NORs are a

site for the formation of nucleolus. From this it is possible to determine the number of NORs based on the number of satellites. In general, the present work has contributed useful information about chromosomal cytology of *Aloe* species in general and endemic *Aloe* species in particular and can serve as a starting point for further and deeper study on *Aloe* chromosomes.

7.2. Recommendations

Based on the present result the following are recommended:

1. Similar work has to be extended to other endemic *Aloe* species in order to document their karyotypes, nucleoli and satellites.
2. A study that involve additional cytogenetical techniques such as C-banding and *in situ* hybridization (FISH) should be carried out to reveal more information about the chromosomes of *Aloe* species.
3. Interspecific hybridization (where possible) and meiotic analysis of the F1 hybrid has to be carried out in order to reveal if there are genomic differentiations beyond the gross morphological similarity of the karyotype of *Aloe* species.
4. In order to better understand the genetic diversity and phylogenetic relationship among *Aloe* species, biochemical and molecular studies are recommended.

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Appendix 1: Chromosome measurements, arm ratios and position of centromere in *Aloe friisii*.

Magnification: 2200

Image resolution: 78.74 pixels per cm

chromosome	Length each		Long arm	Short arm	Arm Ratio (L/S)	Position of centromere
1	13.67829	210.075	10.2412	3.437083	2.97962	sm
2	13.30218	204.2986	9.651183	3.651001	2.643435	sm
3	13.25843	203.6268	10.44929	2.809136	3.719753	st
4	13.10058	201.2025	10.69952	2.401071	4.456144	st
5	13.0752	200.8125	10.75962	2.315581	4.646617	st
6	13.03341	200.1707	10.62749	2.405923	4.417217	st
7	11.49875	176.6011	9.300564	2.198194	4.231003	st
8	11.46226	176.0407	9.190264	2.271997	4.045017	st
9	4.911197	75.42755	3.175531	1.735666	1.829575	sm
10	4.891532	75.12553	3.351173	1.540359	2.175579	sm
11	4.699349	72.17393	3.140709	1.55864	2.015033	sm
12	4.698704	72.16402	2.886947	1.811757	1.593451	m
13	4.368342	67.09023	2.953138	1.415204	2.086722	sm
14	4.24467	65.19083	2.68603	1.55864	1.723317	sm

Appendix 2: Chromosome measurements, arm ratios and position of centromere in *Aloe otallensis*

Magnification: 2200

Image resolution: 78.74 pixels per cm

chromosome	Length each		Long arm	Short arm	Arm Ratio (L/S)	Position of centromere
1	14.23679	216.5953	10.7554	3.481397	3.089391	st
2	13.95646	212.3304	11.06952	2.886947	3.834335	st
3	13.69978	208.4253	11.59847	2.101309	5.519642	st
4	13.28701	202.1455	10.89498	2.392033	4.554694	st
5	13.0312	198.2536	9.823303	3.207898	3.062224	sm
6	12.77762	194.3958	10.68585	2.091772	5.108518	st
7	12.65664	192.5551	10.23209	2.42455	4.2202	st
8	12.54535	190.8621	10.57923	1.966124	5.380752	st
9	4.604063	70.04514	3.525627	1.078437	3.269202	st
10	4.331416	65.89716	3.58096	0.750456	4.771712	st
11	4.299189	65.40686	3.237884	1.061305	3.05085	sm
12	4.075318	62.00094	3.234795	0.840523	3.848548	st
13	4.051866	61.64415	2.89156	1.160306	2.492067	sm
14	3.907168	59.44274	2.604779	1.302389	2	sm

Appendix 3: Chromosome measurements, arm ratios and position of centromere in *Aloe tewoldei*

Magnification: 2200

Image resolution: 78.74 pixels per cm

chromosomes	Length each	Normal length	Long arm	Short arm	Arm Ratio (L/S)	Position of centromere
1	12.15874	204.8013	8.962818	3.195929	2.804448	sm
2	12.05217	203.0061	8.817374	3.234795	2.725791	sm
3	11.95224	201.3229	9.447913	2.504331	3.77263	st
4	11.84577	199.5295	9.180289	2.66548	3.444141	st
5	11.26966	189.8255	8.786709	2.482949	3.53882	st
6	11.19649	188.593	9.155512	2.040971	4.48586	st
7	11.08737	186.7551	8.833047	2.254327	3.918264	st
8	10.78736	181.7017	8.833988	1.953371	4.522433	st
9	4.55308	76.69184	3.22034	1.33274	2.41633	sm
10	4.533537	76.36266	3.073136	1.4604	2.104311	sm
11	4.491468	75.65405	3.011799	1.479669	2.035454	sm
12	4.309279	72.58526	2.866094	1.443185	1.985951	sm
13	4.289388	72.25023	2.842744	1.446644	1.965061	sm
14	4.210467	70.92088	2.600297	1.61017	1.614921	m

Appendix 4: Chromosome measurements, arm ratios and position of centromere in *Aloe camperi*.

Magnification: 2200

Image resolution: 78.74 pixels per cm

chromosome	Length each	Normal length	Long arm	Short arm	Arm Ratio (L/S)	Position of centromere
1	15.82874	219.4277	12.12293	3.70581	3.271331	st
2	15.32726	212.4759	11.46815	3.859109	2.971709	sm
3	14.69261	203.678	11.80105	2.89156	4.081206	st
4	14.24022	197.4067	11.24618	2.994043	3.756185	st
5	13.93051	193.1133	11.23519	2.695319	4.16841	st
6	13.74444	190.5338	11.1269	2.617541	4.250897	st
7	13.65017	189.227	10.90705	2.743114	3.976157	st
8	13.42853	186.1546	10.85659	2.571947	4.221155	st
9	5.40372	74.90968	3.58096	1.82276	1.964581	sm
10	5.184784	71.87465	3.350178	1.834605	1.826103	sm
11	5.091632	70.58332	3.351173	1.740459	1.925453	sm
12	4.941504	68.50215	3.298553	1.64295	2.007701	sm
13	4.843756	67.14711	3.268105	1.575651	2.074129	sm
14	3.965068	54.96619	2.99738	0.967687	3.097469	st

Appendix 4: Chromosome measurements, arm ratios and position of centromere in *Aloe yavellana.*

Magnification: 2200

Image resolution: 78.74 pixels per cm

Chromosome	Length each	length	Long arm	Short arm	Arm Ratio (L/S)	Position of centromere
1	10.75743	212.2899	8.152655	2.604779	3.129884	st
2	10.4555	206.3315	8.485141	1.970357	4.306398	st
3	10.08721	199.0636	8.024315	2.062896	3.889829	st
4	10.05093	198.3476	7.221696	2.829231	2.55253	sm
5	9.916503	195.6948	7.870639	2.045864	3.847098	st
6	9.705429	191.5294	7.562507	2.142923	3.529062	st
7	9.129235	180.1587	7.224231	1.905004	3.792239	st
8	9.018565	177.9747	7.195806	1.82276	3.947752	st
9	4.015133	79.23567	2.571948	1.443185	1.782134	sm
10	3.998275	78.90298	2.550479	1.447795	1.76163	sm
11	3.811408	75.2153	2.349866	1.461541	1.6078	m
12	3.628733	71.61036	2.309817	1.318916	1.751299	sm
13	3.42181	67.52687	2.209534	1.212275	1.822634	sm
14	3.350448	66.11861	2.173036	1.177412	1.845603	sm

Appendix 5: Chromosome measurements, arm ratios and position of centromere in *Aloe harlana*

Magnification: 2200

Image resolution: 78.74 pixels per cm

Chromosome	Length each		Long arm	Short arm	Arm Ratio (L/S)	Position of centromere
1	16.42476	227.0098	13.00712	3.417637	3.805882	st
2	15.66124	216.4572	11.84032	3.820926	3.098809	sm
3	15.14334	209.2991	12.01913	3.12422	3.84708	st
4	14.71822	203.4235	11.24497	3.473252	3.237591	st
5	14.13236	195.3262	11.38986	2.742506	4.153083	st
6	14.0877	194.7089	11.4874	2.600297	4.417728	st
7	13.58196	187.719	11.07563	2.506326	4.419071	st
8	12.07472	166.8871	10.18376	1.890958	5.385503	st
9	5.278172	72.95066	3.452672	1.825501	1.891356	sm
10	5.167886	71.42638	3.431261	1.736626	1.97582	sm
11	4.684155	64.74063	3.24097	1.443185	2.245707	sm
12	4.646869	64.2253	3.061729	1.58514	1.93152	sm
13	4.641267	64.14786	3.179726	1.461541	2.175599	sm
14	4.462598	61.67845	2.886947	1.575651	1.832225	sm

Appendix 6: Chromosome measurements, arm ratios and position of centromere in *Aloe schelpei*
Magnification: 2200

Image resolution: 78.74 pixels per cm

Chromosome	Length each		Long arm	Short arm	Arm Ratio (L/S)	Position of centromere
1	12.01207	209.233	9.638921	2.37315	4.061656	st
2	11.51939	200.6512	8.833982	2.685409	3.289622	st
3	11.48819	200.1077	9.213996	2.274195	4.051541	st
4	11.38776	198.3583	7.771146	3.616611	2.148737	sm
5	11.15852	194.3653	8.945973	2.212549	4.043288	st
6	11.04515	192.3906	8.598708	2.446443	3.51478	st
7	10.32282	179.8087	8.023853	2.298971	3.490193	st
8	9.796246	170.6365	8.285376	1.51087	5.483844	st
9	4.98813	86.88603	3.103351	1.884779	1.646533	m
10	4.689511	81.68453	2.550479	2.139032	1.192352	m
11	4.556182	79.36213	3.001824	1.554357	1.931232	sm
12	4.473855	77.9281	3.048093	1.425761	2.13787	sm
13	3.748758	65.29796	2.194401	1.554357	1.411774	m
14	3.633481	63.29	2.12261	1.51087	1.404893	m