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CHROMOSOME STUDY OF SIX *VERNONIA* SPECIES (ASTERACEAE) FROM CENTRAL ETHIOPIA

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ABSTRACT

*Chromosomes of six **Vernonia** species of the family Asteraceae collected from central part of Ethiopia were studied. The chromosomes were prepared from actively growing root tips by conventional air-dry technique and stained in Giemsa stain. Chromosome counts were made from well spread metaphase plates. All the counts are from somatic cells so that the chromosome numbers are 2n numbers. Chromosome counts of 2n = 20 for **Vernonia amygdalina**, **Vernonia adoensis**, **Vernonia myriantha** and **Vernonia schimperi** and 2n = 18 for **Vernonia leopoldii** and **Vernonia galamensis** were recorded. The karyotypes of the species are more of symmetrical with many median, some submedian chromosomes and only one pair of subterminal chromosomes in **Vernonia myriantha**. Prometaphase chromosome showed chromosomes with satellite (secondary constriction). The numbers of satellited chromosomes observed were one pair in **Vernonia myriantha**, **Vernonia schimperi**, **Vernonia leopoldii** and **Vernonia galamensis** and two in **Vernonia amygdalina** and **Vernonia adoensis**. The results of this study agree with most of the previous studies on the genus.*

Keywords: Chromosomes, Karyotype, Vernonia

1 INTRODUCTION

1.1 Vernonia Species

1.1.1 Taxonomy

The genus *Vernonia* Schreb is a member of large tribe vernonieae in the family Asteraceae (Compositae), order Asterales, class Magnoliopsida and division Anthophyta (flowering plants or angiosperms). The family Asteraceae solely represents the order Asterales. Asteraceae is one of the largest families of vascular plants, consisting of about 1100 genera and over 20,000 species (Cronquist, 1988; Fregonezi *et al.*, 2004). There are about 133 genera and 440 species of Asteraceae family in Flora of Ethiopia and Eritrea. The genus *Vernonia* is represented by about 500 species all over the world and 49 species in Flora of Ethiopia (Mesfin Tadesse, 2004).

The taxonomy of *Vernonia* is recently revised and there are nomenclatural changes and reclassification of some species under other related genera (Isawumi, 1995; Mesfin Tadesse, 2004). *Vernonia* is a complex group from the viewpoint of taxonomy (Angulo and Dematteis, 2009). Cytological data, which is very scarce for this genus, is important in the taxonomy of such a diversified genus in order to maintain the currently assigned taxa or to reclassify them (Oliviera *et al.*, 2006). However, species in this study are identified according to information in the Flora of Ethiopia and Eritrea, Volume 4, part 2 (Mesfin Tadesse, 2004).

All the members of the genus *Vernonia* are characterized by the presence of phytochemicals, sesquterpene lactones (Isawumi, 1995).

1.1.2 Ecology and distribution

The genus *Vernonia,* under the tribe Vernonieae, has large number of species and is found mainly in tropical and tropical and subtropical parts of North and South America, Africa, Madagascar and Southeast Asia (Mesfin Tadesse, 2004). Most of the *Vernonia* species grow in various habitats, reverine, forest margins, acacia woodlands, etc. (Wattmah, 1990). Some of the species grow well in areas that receive little annual rainfall, as low as about 200 mm (Ali *et al.*, 1999). They are found distributed in areas

with altitude range from 1600 m above sea level to over 3000 m above sea level (Mesfin Tadesse, 2004).

1.1.3 Growth forms

Members of the genus are of diverse habit and growth form (morphology) (Agulo and Dematteis, 2009). There are species, which are herbs, shrubs, climbers and trees (Compton, 1976). Ayodele (1999) reported five growth forms among *Vernonia* species in Nigeria, which are erect trees, erect woody shrubs, woody staggering shrubs, erect annual herbs and perennial herbs.

1.1.4 Economic importance

Different species of the genus *Vernonia* have wide range of uses. Some of the species are used as medicinal plants (Schlage *et al.*, 2000), some are used as live fence, dead fence and firewood (Muandu and Tengnas, 2005), and still some others are potential sources of chemicals of industrial importance (Thomson *et al.*, 1994).

The majority of the world's population relies on traditional medicine to some extent for basic healthcare (Biser, 1998). The use of plant materials by people living near the areas where the plants grow is a common practice in Africa and Asia. The use of many *Vernonia* species for treatment of illnesses is recorded from Tanzania, Nigeria, China and Ethiopia (Christina *et al.*, 2000; Huang *et al.*, 2003; Ojiako and Nwanjo, 2006; Tilahun Teklehaymanot and Miruts Giday, 2007). Chimpanzee suffering from diarrhea and malaise ate succulent pith of young shoot of *Vernonia amygdalina* and recovered within 24 hours and started their usual activities (Biser, 1998). People in southwest China use parts of *Vernonia saligna* for treatment of sore throat, cough, tuberculosis and uterus prolapse (Huang *et al.*, 2003). *Vernonia amygdalina* is used in folk medicine of Nigeria to cure malaria and helminthes infection (Ojiako and Nwanjo, 2006).

Different *Vernonia* species are used in Ethiopian traditional medicine. The root of *Vernonia adoensis* is chewed with honey for treatment of menstrual disorders and leaf extract of *Vernonia amygdalina* is orally administered to treat tonsillitis by the people of Zegie peninsula, Northwest Ethiopia (Tilahun Teklehaymanot and Miruts Giday, 2007).

The traditional use of *Vernonia amygdalina* (locally known as Girawa, Amh. Ebecha Oro.) leaf extract as an antifertility agent in Ethiopian traditional medicine is tested experimentally on mice by Chirotaw Ayele (2006) and the result is in agreement with the traditional use. The use of *Vernonia amygdalina* as tick control by people of western Ethiopia was reported by Regassa (2000).

Following these traditional therapeutic uses of *Vernonia* species, many scientific investigations have been conducted on the phytochemistry and pharmacological activities of the plants. A study done in South Africa showed that extracts from leaves of *Vernonia myriantha* have high antiplasmoidal (antimalarial) activity *in vitro* (Clarkson *et al.*, 2004). Extract of *Vernonia amygdalina* leaves was also found to have hepatoprotective potential by *in vivo* study on mice (Iwalokum *et al.*, 2006; Ojiako and Nwajo 2006). Extrat of *Vernonia cinera* was also experimentally tested and proved to have antinfective activity (Huang *et al.*, 2003).

Different studies on *Vernonia amygdalina* show that it has potential natural product for treatment of various disorders. Its leaf extract was tested for its anticancer activity on mice and was found to be a good candidate for cancer therapy (Yedjon *et al.*, 2008) and it was also found to reduce cholesterol induced hyperlipidemia (Adramaye *et al.*, 2008).

In addition to its use in treatment of human and animal diseases, extract from *Vernonia amygdalina* was found to have good potential in preventing plant fungal diseases, which highly damages many of crop plants (Alabi *et al.*, 2005). In many parts of Ethiopia, leaves of *Vernonia amygdalina* are used traditionally in washing pots used for preparation of homemade beverage "Tella" (Mesfin Tadesse, 2004).

In Nigeria and other tropical African countries, *Vernonia amygdalina* is used as leafy vegetable. It supplements weaning foods by essential proteins, vitamins and minerals. It also has appetizing and febrifuge effects. *Vernonia amygdalina* is also a well known animal feed in Nigeria (Ojiako and Nwanjo, 2006), and though it is not cultivated as animal feed, cattle eat its leaves from naturally growing plants in Ethiopia also.

Vernonia amygdalina was also found to have industrial importance as its ethanol extract showed corrosion inhibition activity in mild steel (Odeongenyi *et al.*, 2009).

Vernonia galamensis is attracting the attention of industrial research due to its epoxy oleic acid and vernolic acid content. Natural epoxy acid is used in paint industry as a substitute for plastisizers and stabilizers (Trumbo *et al.*, 1999). Vernolic acid and epoxy oleic acids were first discovered in *Vernonia anthelmentica* (L) Willd in India in 1954. Later *Vernonia galamensis* was found to contain better vernolic acid than any improved variety of *Vernonia anthelmentica* (Wattmah, 1990). Different studies show that the seeds of *Vernonia galamensis* have oil content of more than 40% (Ali *et al.*, 1999). A recent work by Getachew Ali (2008) on Ethiopian varieties of *Vernonia galamensis* shows average oil content of 35.21% with high variability among various accessions.

Vernonia galamensis, as a potential oil crop, has several advantages over many other oil seeds. First it has high oil yield which is more than 40% oil content of total seed weight, 80% of which is vernolic acid (Ali *et al.*, 1999). Second, the plant is resistant to insect pests, disease and drought (Persinos, 1992) and has relatively higher seed yield per plot of land (Thompson *et al.*, 1994). Third, *Vernonia galamensis* grows in areas with low annual rainfall as little as 200mm of seasonal rainfall (Ali *et al.*, 1999). This makes it suitable cash crop for farmers of arid and semi arid areas (Thompson *et al.*, 1994; and Tesfaye Baye and Sileshi Gudeta, 2002).

Other industrial products of *Vernonia galamensis* are degradable lubricants and lubricant additives, epoxy resins, adhesives, insecticides and insect repellants, crop oil concentrates and formulations of slow release pesticides (Ali *et al.*, 1999).

These days, environmental concern is growing all over the world, and paint industries use volatile organic compounds (VOC) in paints and coatings. VOCs are among the major pollutants that contribute to the global warming (Persinos, 1992; Trumbo *et al.*, 1999). The natural epoxy fatty acids from *Vernonia* are better substitutes in paint industries

because of their low VOC and environment friendly products (Thompson *et al.*, 1994; Trumbo *et al.*, 1999). According to Trumbo *et al.* (1999), *Vernonia* oil also has suitable viscosity for use in paints and coatings and it is good solvent and cross-linking agent in reactive solvent system.

1.1.5 Chromosome

According to Dematteis (1998), the genus *Vernonia* is not well characterized cytologically with only about 16% of the species examined for chromosome number by then. Especially, the Old World *Vernonia* are among the poorly addressed taxa regarding chromosome data (Mesfin Tadesse, 2004). In the literature, some New World species have been found studied and chromosome numbers reported, but very little of African and other Old World species have been studied.

Chromosome number reports show that there is variation among *Vernonia* species (Hunter, 1964). Chromosome counts of n = 9, 10 and 20 were recorded from the Old World *Vernonia* species (Hunter, 1964; Jones, 1974; Adegbite and Ayodele, 2004). Counts from the New World *Vernonia* species, specifically those from South America, show that there are species with n = 10, 14, 16, 17, 20, 30 and 34 and some with 2n = 30 and 128 (Dematteis, 2002). Getachew Ali (2008), reported that nine bivalents are observed in meiotic chromosomes of *Vernonia galamensis*, which means 2n = 18. This is the only recent work on chromosomes of Ethiopian *Vernonia* species.

Reports on basic chromosome numbers of the genus show that it varies between x = 7 and x = 19 (Dematteies 1998). The Old World *Vernonia* species fall under two categories of basic chromosome numbers of x = 9 and x = 10 (Ayodele, 1999). Though basic number x = 17 is frequently found in South American species, there are basic numbers of x = 10, 14, 15, 16 and 19 suggested for the New World *Vernonia* species (Dematteis, 2002). This shows that the genus is multibasic. This multibasicity may have arisen from complex combination of polyploidy and aneuploidy. The x = 17 basic number present in many South American species may have been derived from x = 9 through duplication to 18 and subsequent reduction by aneuploidy to 17 (Angulo and Dematteis, 2009). This assumption is derived from the suggestion of Jones (1974) that the ancestral basic

chromosome number in the genus is x = 9 or x = 10. Mathew and Mathew (1976, 1982) postulated the x = 10 to be a primitive chromosome number and the x = 9 could have evolved by aneuploidy reduction. The fact that high number of species with x = 9 being aggressive colonizers while species with x = 10 are poor colonizers, seem to support this postulate (Ayodele, 1999). Another view on the basic chromosome number of the genus suggests that, *Vernonia* has evolved from closely related ancient species with x = 4 and x = 5 by mechanisms such as hybridization, polyploidization and aneuploidy decrease (Adegbite and Ayodele, 2004).

The other aspect of karyotype study is the ploidy level of the species. According to Dematteies (1998), polyploidy is common in the New World *Vernonia* species whereas the Old World species do not exhibit significant polyploidy.

Some works on both the Old and New World *Vernonia* species indicated the karyotype symmetry of the studied species. Dematteis (1998) showed that the New World species tend to have symmetrical karyotype. Ayodele (1999), in his work on species from Nigeria, mentioned that there is no symmetry in sizes of the chromosome and even different species show different degree of asymmetry in their karyotypes.

1.2 Chromosome Study

From the time they were first observed, chromosomes have been studied in different ways for different purposes, from clinical diagnosis to basic research (Speincher and Carter, 2005). One of the most common reasons for chromosome study is to determine the identity of organisms (De Melo *et al.*, 2000). Chromosomes have been used to assign organisms to different taxa as members of the same species have similarity in their chromosome sets and related species have related chromosome sets (Gill and Singhal, 1998; Stace, 2000). Information from chromosome study is also useful in predicting reproductive property and/or fertility of organisms (Stace, 1991). Many health defects and genetic disorders are also diagnosed by examining the chromosomes of individual organisms. If individuals differ in their chromosomal features from other members of the species, it is considered to carry certain disorder (Speicher and Carter, 2005).

Techniques of examining chromosomes also vary from conventional staining to molecular cytogenetic techniques (Speicher and Carter, 2005). Chromosomes from actively dividing cells are prepared and stained by different chromosome specific dyes such as feulgen staining technique to study their general features (Gill and Singhal, 1998). Detailed chromosomal analysis is done by various chromosome banding techniques which were developed in 1970s and are still used in many researches (Speicher and Carter, 2005). More advanced techniques recently in high utilization by researchers are *in situ* hybridization (Sadder and Webber, 2001). In these techniques, DNA sequences complimentary to regions on chromosomes are tagged with fluorescent dyes and are allowed to anneal to the chromosomes, resulting in characteristic patterns of fluorescent patches on the chromosomes (Fregonezi *et al.*, 2004). *In situ* hybridization techniques are based on molecular concept that, complementary nucleotide sequences could hybridize, and these techniques are sometimes known as molecular cytogenetic techniques (Speicher and Carter, 2005).

With all these techniques, the main features of chromosomes to be studied are the chromosome numbers, morphologies and behaviors. For the number and morphologies, mitotic chromosomes are appropriate materials and for chromosome behaviors (pairing and distribution during cell division), meiotic chromosomes are appropriate (Gill and Singhal, 1998).

1.2.1 Chromosome number

All cells of an individual organism have similar set of chromosomes. It is well known that this set of chromosomes with specific number and right combination is necessary for the survival or at least well-being of an organism. The work of Boveri at the onset of the 20th century, showed that for an organism to grow and live normally, chromosome set in the parent organism(s) must be maintained not only in numbers but also in composition (Sturtevant, 2001). This conservation of specific chromosome sets in all cells extends beyond individual organisms and all members of species having a characteristic chromosome number in all their somatic cells (Stace, 1991). In some taxa, this identity of chromosome number goes beyond the species level and all members of the genus may have the same chromosome number (Demerico *et al.*, 1995). Even if it varies, in many

cases the numbers will be simple multiple of the number in closely related taxa (Stace, 2000).

As one of the character of taxa, chromosome number is of great importance both in classification and in evolutionary and phylogenetic studies of species (Stace, 2000; Sytsma and Pires, 2001). Most of the time, somatic chromosome numbers in certain taxa are even number multiples of the smallest gametic number. This condition makes the small number the character of the taxon. As chromosome numbers of all the members of the taxon are based on this number, it is known as basic chromosome number of the taxon (Gill and Singhal, 1998). However, determining basic chromosome number of a taxon is not an easy task, because the numerical variation of the chromosomes is not only the result of polyploidization, but also there are other factors that change the chromosome number in unpredictable way (a way that does not result in multiple of the basic number) (Stace, 2000).

Chromosome number in plants has long been documented, one of the oldest records found being that of Strasburger (1882; cited in Stace, 2000). By the end of the 20th century, chromosome numbers were reported for only about 25% of angiosperm species (Stace, 2000). According to Stace (2000), the chromosome number reports cover only some areas of the world (plants from all over the world are not equally represented in the reports). Although chromosome count is not done so much these days, the systematists are revisiting the preexisting data on chromosome numbers for it provides information on taxonomic position of the organism or the taxon (Sytsma and Pires, 2001).

1.2.2 Chromosome morphology

What matters for the survival of an organism is not only the chromosome number but also it is the appropriate composition of the chromosome set. This means that each individual chromosome has its own role in the survival and development of the organism (Sturtevant, 2001). As chromosomes vary in function, they may also vary in their morphology as was first noted by Sutton (1902) cited in Sturtevant (2001). This morphological difference makes chromosome sets fingerprint of the species because the survival of the organism is determined by the presence of complete set(s) of

chromosomes in which each individual chromosome has its own function and structure (Sytsma and Pires, 2001). The morphological variation together with the numerical differences of the chromosome sets adds value to chromosomes to be used as taxonomic tool, because whenever there is similarity in number among different taxa, they may be differentiated by the morphology (Demerico *et al.*, 1995). Among the major morphological features of chromosomes are the size of the chromosome, the centromeric position and absence or presence of secondary constrictions and associated satellites and their position are among the major ones (Jackson, 1971).

1.2.2.1 Chromosome size

One of the chromosome's gross morphologies is its size, more appropriately the length. The length of chromosomes is directly proportional to the amount of DNA it contains (Stebbins, 1971). Measuring the size of a chromosome is not easy because of differences in degree of condensation, which affects the length of chromosomes. Although it is difficult to measure chromosomes' absolute size, other measurements such as relative size are used to characterize each chromosome. Relative chromosome size can be expressed by taking one chromosome as standard and the length of others is expressed relative to it (Stace, 1991). The other relative size measurement is by calculating the ratio of each individual chromosome lengths to the total length of the chromosomes in the set (Chang *et al.*, 2004; Gomurgen and Altiozlu, 2005). Both absolute and relative chromosome sizes are important in characterizing the chromosome set of the individual organisms and/or the taxa (Lavania and Srivastava, 1992; Agbagwa and Okoli, 2005; Adetula, 2006). In addition to identity, chromosome size has great importance in chromosome evolution because the size of chromosome affects the way they behave in cell division. Since the absolute chromosome size affects the total genome size, this also affects the tolerability of polyploidy as the nuclear material needs to be packaged efficiently in the nucleus (Stebbins, 1971). Longer chromosomes tend to be frequently rearranged and recombined because of formation of many synaptonemal complexes than the shorter ones and this leads to change both in chromosome morphology (by rearrangement) and number (by polyploidy) due to presence of more synapsis (Sites and Reed, 1994). Increased polyploidy in other ways reduces fertility and may lead to sterility (Sumner, 2003).

1.2.2.2 Centromeric position

Centromere or primary constriction is a constricted region that appears on metaphase chromosomes and is the site of spindle attachment during cell divisions. Attachment of spindle microtubules to the centromere is through the formation of kinetochore complex at the centromere (Lamb *et al.*, 2007). This makes the centromere fundamental chromosomal structure in all cell divisions (Malik and Bayes, 2006). According to Stace (2000), in some plants there are chromosomes without such a constriction, but undergo normal segregation during cell divisions. Such chromosomes were once called acentric because they seem to lack centromere. Later it was discovered that the kinetochore (kinetic activity) is found diffused along the length of the chromosome and these chromosomes are called polycentric /holocentrics/ (Stace, 2000; Malik and Bayes, 2006). The usual types of chromosomes have one centromeric region and are called monocentric. In monocentric chromosomes, when segments without centromere or acentric fragments of chromosomes are formed they will be lost during cell division, because they will not be recruited to the daughter cells by spindles (Pierce, 2006).

In chromosome studies, centromeric position is the most commonly used morphological feature of chromosomes (Stace, 2000). In some cases, by chance, both chromosome number and size may appear to be the same. In such cases, the position of the primary constriction (centromere) is used in discriminating chromosome sets of different taxa (Gill and Singhal, 1998). Different morphological parameters of chromosomes are calculated based on the position of the centromere, which characterize both individual chromosomes and chromosome sets or karyotype of the organism. Among these are centromeric gradient, which is the ratio of the length of the short arm to the length of the chromosome and dispersion index, which is calculated based on centromeric gradient (Lavania and Stivastava, 1992). Centromere is used as a landmark to measure chromosome arms, and hence to describe many morphological indices of chromosomes (Begum *et al.*, 2007). Other common parameters such as arm length, arm ratio, centromeric index, and relative length of arms are calculated based on the position of this primary constriction (Levan *et al.*, 1964; Fregonzi *et al.*, 2004; Gomurgen and Altinozlu,

2008). Levan *et al.* (1964) had categorized and named chromosomes as median point, median region, submedian region, subterminal region, terminal region and terminal point, designated as M, m, sm, st, t and T, respectively, depending on their arm ratio, which is calculated based on the centromeric position.

Centromeric position has long been used to designate chromosome types. Chromosomes with centromere at one end (chromosome with a single arm) are termed telocentric, those with centromere near one end (with one long arm and one short arm) are called acrocentric , those with centromere closer to the middle than acrocentric chromosomes but not at the middle (the length of the two arms distinctly different) are sub-metacentric and those with their centromere at the middle of the chromosome (with two equal arms) are metacentric (Stebbins, 1971). However, such classification of chromosomes is subjective, especially the distinction between sub-metacentric and acrocentric chromosomes is not sharp. The work of Levan *et al.* (1964) solves the problem because the classification is determined by calculated numerical values (centromeric position) so

Table 1 Terminologies for chromosomes with respect to centromeric position

Term	Location	d value	r value
M	Median point	0	1
m	Median region	0.0 - 2.5	1.0 - 1.7
sm	Sub-median region	2.5 - 5.0	1.7 - 3.0
st	Sub-terminal region	5.0 - 7.5	3.0 - 7.0
t	Terminal region	7.5 - 10.0	7.0 - ∞
T	Terminal point	10	∞

Source; Levan *et al.*, 1964.

The d value is calculated by dividing the whole length of the chromosome in 10 equal units.

that allocation of chromosomes to certain class is not by personal judgment. The position of the centromere can be expressed in terms of arm ratio (ratio of the long arm to the short arm) (r) or length difference (d) between the long and short arms (Table 1).

1.2.2.3 Secondary constriction and chromosome satellites

Constricted regions on metaphase chromosomes, other than primary constriction (centromere) are known as secondary constrictions (Luke *et al.*, 1992). These regions of chromosomes are highly heterochromatic regions between chromosome satellites and the rest part of the chromosome (Stace, 2000). Some authors call these regions nucleolar-organizing regions (NORs) because they are sites of DNA that code for ribosomal RNAs (rRNAs) and rRNAs are transcribed in nucleoli (Stace, 2000; Sumner, 2003). However, some works show that nucleolar organizing activity and secondary constriction are neither associated nor unrelated (Berns and Cheng, 1971). According to Sato *et al.* (1980), not all nucleolar organizing regions are indicated by the presence of secondary constriction, in which case chromosome satellites may not be formed.

As chromosome morphological structure, secondary constrictions are among the important karyological features because it determines the chromosome satellites. The number of chromosomes bearing secondary constriction (satellited chromosomes) and the position of satellites on the chromosome arms are among the distinguishing characteristics that are observed from karyogram of an organism (Guerra *et al.*, 1997). In addition, these regions are of great importance in chromosome morphological changes as they are prone to breakage and inversion (Luke *et al.*, 1992).

The presence and number of secondary constrictions is not consistent in all mitotic preparations of an organism. This inconsistency is perhaps due to metabolic activity (non-involvement of the chromosome in nucleolar organizing) in the previous interphase or they escape detection due to weakly stained or destained regions on chromosomes. They may also disappear (become condensed) when the chromosome is highly condensed. The number, site and distribution of the nucleolus organizing region is reliably determined by *in situ* hybridization with rDNA probes rather than from secondary constriction (Stace, 2000).

1.2.3 Chromosome evolution

Both number and structure of chromosomes are subject to change due to different reasons and with various consequences. Many changes in chromosome number and morphologies

result in diverse disorders among which many are fetal (Fadl-Elmuna, 2005; Griffiths *et al.*, 2008). In some cases, changes in chromosomes, which can be detected by chromosomal examination, are not associated with health defects (Kowaleyk *et al.*, 2007). When such harmless changes occur to chromosomes and are fixed in a certain population, it is considered evolution at chromosomal level (Sites and Reed, 1994).

1.2.3.1 Changes in chromosome numbers

Chromosome number may change in various ways and results in a new chromosome set which has effect on the general biology of the organism (Schubert, 2007). The most common numerical changes are generally categorized into two. Changes due to loss or gain of complete set(s) of chromosomes are categorized as aberrant euploidy and loss or gain of only part of complete set fall under general class called aneuploidy. Aberrant euploids can be monoploids- where a diploid organism loses a haploid set of chromosomes or polyploids- where more than two sets of basic number of chromosome per cell exists, polyploidy being the most common of all. Aneuploids can be trisomics, monosomics or nulsomics (Griffiths *et al.*, 2008). When looked at a particular homologue chromosome, aneuploid change in chromosome numbers may also occur due to fusion or fission of chromosome arms which also affects chromosome morphologies (Schubert, 2007; Change *et al.*, 2008).

Polyploidy is the commonest of all changes in chromosome number, especially in plants (Stace, 2000). This increase in chromosome number by complete set is of two types based on the origin of the additional chromosome set. It is called autopolyploidy if the added chromosome set is from the same species and allopolyploidy if the chromosome set added is from different species (Griffiths *et al.*, 2008). Allopolyploidy and repeated polyploidization processes make the numerical change diverge from simple multiple of one specific haploid number.

In some plants, the level of ploidy is thought to reach up to 84 ploidy (the case of a fern, *Ophioglossum reticulatum* whose 2n = 1260 and base number of x = 15). However, in some cases high level of polyploidy reduces fertility and may lead to sterility (Stace, 2000).

Polyploidy is also advantageous for it is a source for new species of plants or new genetic makeup and also useful for polyploids often have more advantageous characteristics that the diploids do not have (Griffiths *et al.*, 2008).

Aneuploidy as a source of genetic variation plays an important role in changing the chromosome number of species and creating new chromosome set (Agbagwa and Okoli, 2005). Modification of chromosome number by addition or loss of few chromosomes is better tolerated in plants, especially the polyploid ones than in animals (Griffiths *et al.*, 2008).

1.2.3.2 Chromosome structural change

Changes that affect the morphology of chromosomes may arise from various cellular phenomena. The main mechanisms of chromosome structural evolution are the different types of chromosomal rearrangements and deletions (Jones, 1998; Natarajan, 2002; Schubert, 2006) and fusion and fission of chromosome arms (Change *et al.*, 2008).

Deletions or deficiencies. Deletions or deficiencies refer to loss of block of chromatin from a chromosome (Swanson, 1967). The part deleted may range from only few base pairs (microdeletion) to full chromosome arm or the whole chromosome (Coghlan *et al.*, 2005). Deletion may result in change in structure of chromosome and even may change chromosome number in cases where full chromosome is deleted. Deletion reduces both chromosome size and the total genome size. Depending on the region of chromosome deleted, the position of the centromere may be changed from the middle of the chromosome to the terminal or vice versa. According to Sharma and Sharma (1959), deletion of chromosome segments together with other chromosomal rearrangements play important role in karyotype evolution.

Duplication. Occurrence of extra copy of chromatin segment (duplication) in chromosome is another factor that contributes to chromosome structural change. Duplication may be direct tandem, where the extra copy is adjacent to and is in the same orientation with the original segment, or inverted tandem duplication, where the extra

copy is in reverse orientation with the original copy (Coghlan *et al.*, 2005). Both types of duplication result in increase in chromosome size and genome size and also changes the structure of the chromosome. Duplications are more frequent in nature and are less lethal than deletions (Swanson *et al.*, 1967). Duplication has played important role in evolution of species in primates (Sumner, 2003).

Inversions. When a segment is broken, turned around 180^0 and rejoin, so that the order of genes on the segment is reversed with respect to the order of genes on the rest part it is said to be inverted (Stebbins, 1971). Inversions are of two types depending on whether the inverted segment includes the centromere or not. When the inversion occurs on one arm only (centromere not included), it is called paracentric inversion and when it occurs on the region that contains the centromere (breaks occur on both arms) it is called pericentric inversion (Jackson, 1971).

Inversions do not affect the size of chromosome and genome size as deletions and duplications do. Pericentric inversions can affect the position of the centromereif break points in the two arms are not equally far from the centromere. When major chromosome markers such as secondary constriction are involved in paracentric inversion, it will change the chromosome morphology by altering the position of secondary constriction respectively (Schubert, 2007). In addition to karyotype change, inversions reduce the rate of recombination in genomes where there is inversion polymorphism (Hoffmann *et al.*, 2004).

Translocations or interchanges. Translocations or interchanges are phenomena that occur due to simultaneous breaks and exchange of parts (sometimes chromosome arms) in two unpaired chromosome. The exchanged parts may be equal or unequal. Unequal exchange can easily be detected by conventional karyotype analysis while equal exchange requires advanced techniques (Jackson, 1971; Savage, 1977). There are also translocations of segments within the same chromosome from one location to another (Coghlan, 2005). Translocations alter chromosome structure and even reduce

chromosome number if the chromosome is lost after having translocated its essential parts to other chromosome (Stebbins, 1971).

Centric fissions and fusions. As the name indicates, these phenomena are dissociation and association of chromosome arms. They are considered as types of translocation by some authors when they occur consecutively (Jackson, 1971). Fusion of chromosomes is sometimes referred to as Robertsonian fusion as it was first postulated by Robertson. Centric fission is breakage of chromosomes at centromeric region resulting in two telocentric chromosomes (Sumner, 2003). The fission products may have different fates: 1- they remain independent chromosomes if they fulfill the minimum requirement (Jones, 1998); 2- fission products of two chromosomes may fuse to form two metacentric chromosomes or isochromosomes if sister chromatids reunite (Jones, 1998; Perry *et al.*, 2004) and 3- may be lost from the genome due to lack of sufficient centromere or telomere or due to diminished size (Perry *et al.*, 2004).

Generally, all these factors affect both the number and structure of chromosomes. The consequence of one may be contradicted by the effect of the other phenomena. Deletions reduce the overall length of chromosomes or the genome size, whereas duplication increases the chromosome or genome size. Inversions do not have effect on the size. Fissions increase chromosome number whereas fusions reduce the number. Translocations may affect the size of individual chromosome without affecting the genome size. The effects on the morphology are not directional. Therefore, it is difficult to conclude the evolutionary direction of chromosomal changes (Jones, 1970).

However, some authors analyze the evolution of chromosome morphology by the use of karyotype symmetry, symmetrical karyotype being the one with all chromosomes metacentric and of equal size. According to these authors, karyotypes usually tend to evolve from symmetrical towards asymmetrical karyotype (Stebbins, 1971; Stace, 2000).

1.2.4 Applications of chromosome study

Chromosome study gives a wide range of karyological information by the use of different techniques. Information derived from such studies is applicable in many fields. It is

utilized in diagnosing many human clinical defects ranging from different aneuploidy to cancer (Kirsch-Volders *et al.*, 2002; Speicher and Carter, 2005; Fadl-Elmuna, 2008). Information on gene regulation and expression and other biochemical process is obtained from cytogenetic studies (Tessadori *et al.* 2004). Karyological data are also used in studying evolutionary trends (Balanya *et al.*, 2003), breeding and fertility studies (Gill and Singhal, 1998; Stace, 2000) and is extensively utilized in taxonomy and systematics (Gill and Friebe, 1998; Stace, 2000; Systma and Pires, 2001). These days, techniques for whole genome sequence are available; however, they must be supplemented by karyological data as they will not reveal chromosomal evolution, which is important in many fields of biology (Fraut, 2008).

Information from cytogenetics and specifically from chromosomal studies is potential for future sciences of systematics and evolutionary biology. In systematics, chromosome number, morphologies, size and symmetry are used to show relatedness or divergence of taxa (Stace, 2000). Chromosome basic number is one of the widely used taxonomic tools in systematics. Although there are cases, where karyotypes of related organisms appear to differ widely, advanced cytological techniques can show the interrelatedness and even the types of chromosomal rearrangements that have brought about the differences seen (Jackson, 1971).

Patterns in chromosomal variations most of the time indicate the direction of evolution (Jones, 1970). Most of the chromosomal changes are accompanied by morphological, physiological and genetic changes. Some of such changes are adaptive while some are simple variations. This means both the new and the original karyotype are equally adapted to common ecosystem or the two forms specialized to different ecosystems (Sharma and Sharma, 1959).

Some authors argue that chromosomal changes that occur and fixed in a population may lead to speciation and some others consider the variation as simple polymorphism within a species (Searle, 1998). Chromosomal rearrangements may lead to speciation as it

reduces gene flow through suppression of rearrangements (Riesenberg, 2001), and by creating reproductive barriers (Sumner, 2003).

Generally, differences and similarities that are derived from genome data in general and karyological data in particular strongly suggest taxonomic differences and/or similarities (Mable, 2003; Yi, 2006). Information on chromosome number, genome size, chromosome morphologies and chromosome banding patterns, chromosome hybridization, chromosome behavior and homology is of great importance and is a fundamental resource for taxonomy (Stace, 2000).

2 OBJECTIVES OF THE STUDY

2.1 General Objective

To generate cytological (chromosome) data of some *Vernonia* species found in Ethiopia.

2.2 Specific Objectives

- To document chromosome numbers of the *Vernonia* species under the study,
- To examine the ploidy level of the species, and
- To construct karyotypes of the species studied.

3 MATERIALS AND METHODS

3.1 Plant Materials

Among the 49 species reported to be found in Ethiopia (Mesfin Tadesse, 2004), six were collected from central part of the country. Five of the six species were collected from natural habitats and one (*Vernonia galamensis*) was obtained from previous collection for research(Table 1.). Seeds of the plants were collected and germinated in laboratory and transferred to a greenhouse at Addis Ababa University, Science Faculty, Department of Biology. Voucher specimens were also collected from the natural habitat of the plants and deposited at National Herbarium (ETH), Addis Ababa University.

Table 2. *Vernonia* species studied, number of plants examined, number of cells analyzed, sites and altitudes of collection.

Plant species	Number of plants examined	Number of cells analyzed	Site of collection	Altitude(meters above sea level)
Vernonia amygdalina	8	50	Addis Ababa	2500
Vernonia adoensis	7$^{\Phi}$	24	Arsi, Bulala	ca 2000
Vernonia galamensis	5$^{\Phi}$	20	From previous collection (Getachew Ali, 2008)	-
Vernonia leopoldii	6	22	Menagesha, along the road to Ambo (about 2.5km from Menagesha town)	2400
Vernonia myriantha	12	49	Arsi, Huruta	2000
Vernonia schimperi	7$^{\Phi}$	22	Nazareth town	1600

$^{\Phi}$ Slides prepared from germinating seeds, hence the numbers indicated are the number of slides which may contain cells from more than one seed.

3.2 Chromosome Preparation

Somatic chromosomes were prepared from actively growing root tips excised from seedlings and germinating seeds. Root tips from seedlings were collected in the evening, and from germinating seeds any time of the day. The root tips were treated with 8-hydroxyquanoline for 3-4 hours or kept in ice-cold water for 18-20 hours to inhibit spindle formation. The chemical (8-hydroxyquanoline) is decanted and the root tips rinsed in distilled water and transferred to fixative (ethanol: acetic acid, 3:1) and kept at 4^0C overnight. The root tips were removed from the fixative and rinsed several times with distilled water again before maceration with enzyme (4% cellulase + 4% pectinase) solution at 37^0C. After maceration, the very tips of the roots detach from the rest part of the root. Then the enzyme solution was decanted and the root tips rinsed in distilled water and kept in distilled water for about 15 minutes.

The detached root tips were then taken with a pipette and placed on microscope slides. The water was removed by absorbent tissue paper and the tips mashed in a drop of fixative with spatulated needle and spread with strong air blow. The slides were then air dried at room temperature.

The air dried slide was then stained in Giemsa stain (pH = 6.8) for 45minutes to one hour. Then the slide rinsed and air dried. To make the slides permanent it is mounted in DPX mounting medium and covered with 24mm×50mm coverslip. The slides were then examined with light microscope for metaphase chromosomes and micrographs of well spread metaphase plates were taken. Counts and karyotype analysis were performed from micrographs of well spread chromosomes.

4 RESULTS

Karyological data of six *Vernonia* species collected from different locations of central Ethiopia are presented below. Chromosome counts and karyotype formulae are presented in Table 3. All the six species examined exhibit unimodal karyotype with many median and submedian and only one pair of subterminal chromosomes.

Table 3. Chromosome numbers and karyotype formulae of *Vernonia* species studied

Species	Chromosome number	Karyotype formula[#]
Vernonia amygdalina	20**	6m+14sm
Vernonia adoensis	20	20m
Vernonia galamensis	18	16m+2sm
Vernonia leopoldii	18*	18m
Vernonia myriantha	20*	4m+14sm+2st
Vernonia schimperi	20*	20m

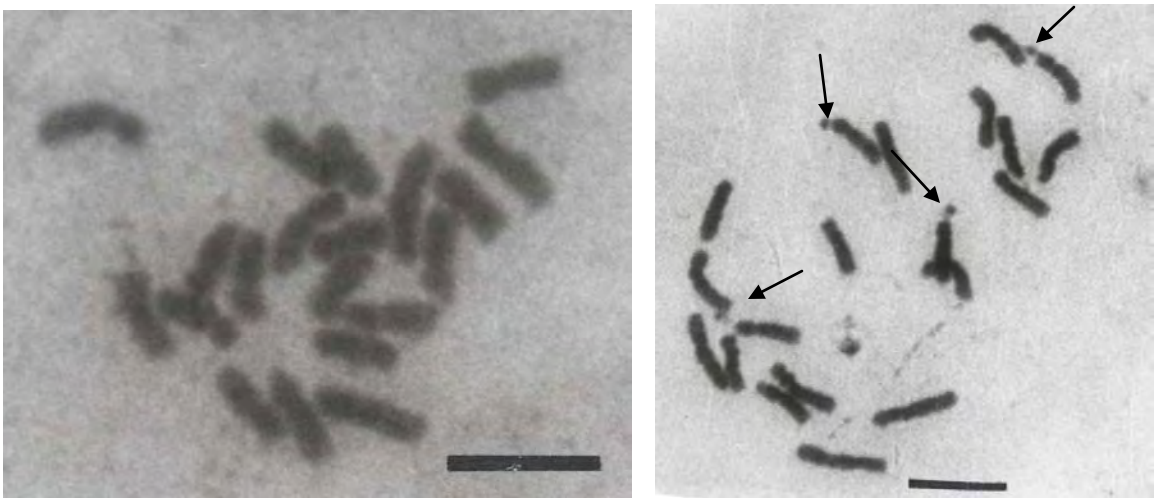
[#] The chromosomes are classified based on the position of the centromere: M = median point, m = median region, sm = submedian region, st = subterminal region, t = terminal region and T = terminal point.

** Chromosome number which differ from previous report.

* Chromosome number reported for the first time.

4.1 *Vernonia amygdalina* Del.

The chromosome number of *Vernonia amygdalina* is 2n = 20 (Table 3 and Figure 1A and B). The karyotype is composed of 6 median and 14 submedian chromosomes (Figure 1C). Two pairs of the chromosomes with satellites (secondary constriction) are observed in prometaphase chromosomes (Figure 1B). The chromosome size ranges from about 4.6µm, the smallest to 7.9µm the largest. The total chromosome length is 125.9µm (Appendix 1).



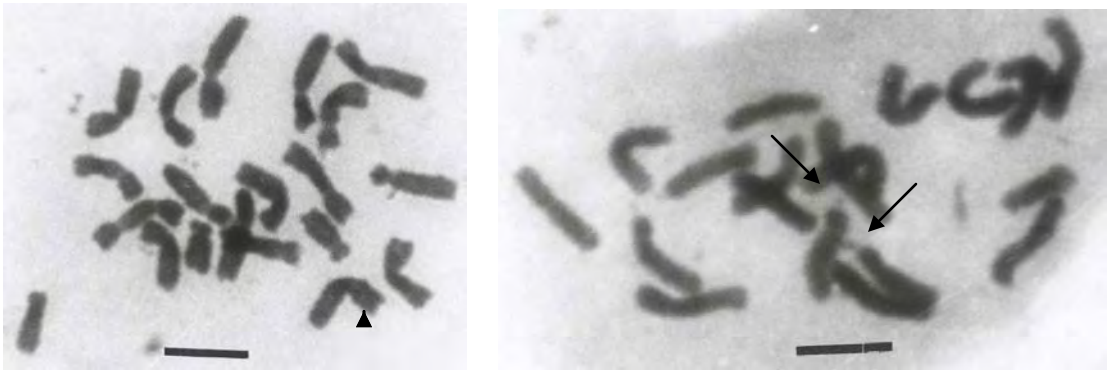
A B

C

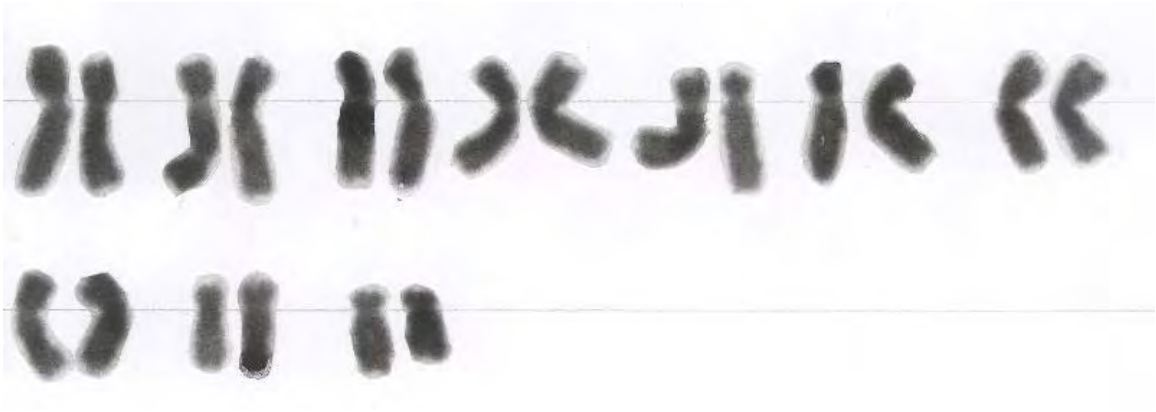
Figure 1 *Vernonia amygdalina* Chromosomes. (A) Metaphase chromosomes, (B) Prometaphase chromosomes to show chromosome satellites (arrows), (C) Karyotype. Scale bars = 10 µm.

4.2 Vernonia myriantha Hook. f.

The chromosome number of *Vernonia myriantha* is 2n = 20 (Table 3 and Figure 2A and B).The karyotype is composed of 4 median, 14 submedian and 2 subterminal chromosomes (Figure 2C). One pairs of the chromosomes with satellites (secondary constriction) are observed in prometaphase chromosomes (Figure 2B). The chromosome size ranges from 6.1µm, the smallest to 11.9µm the largest. The chromosomes of *Vernonia myriantha* are larger compared to the others with total chromosome length of 192.1µm (Appendix 2).



A B

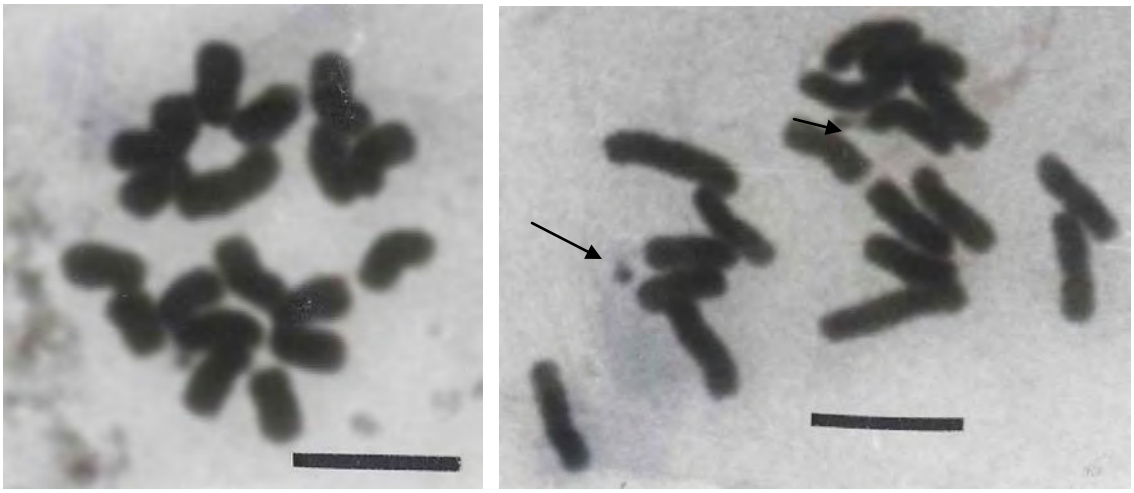


C

Figure 2 *Vernonia myriantha* chromosomes. (A) Metaphase chromosomes, (B) Prometaphase chromosomes to show chromosome satellites (arrows), (C) Karyotype. Scale bars = 10 µm.

4.3 Vernonia leopoldii (Sch. Bip. ex Walp.) Vatke

The chromosome number of *Vernonia leopoldii* is 2n = 18 (Table 3 and Figure 3A and B).The karyotype is composed of 18 median chromosomes (Figure 3C). One pair of the chromosomes with satellites (secondary constriction) is observed in prometaphase chromosomes (Figure 3B). The chromosome size ranges from 3.7µm, the smallest to 6.6µm the largest. Total chromosome length is 82.1µm (Appendix 3).



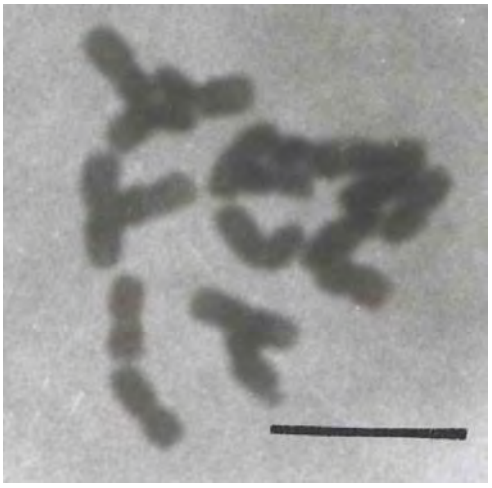
A B

C

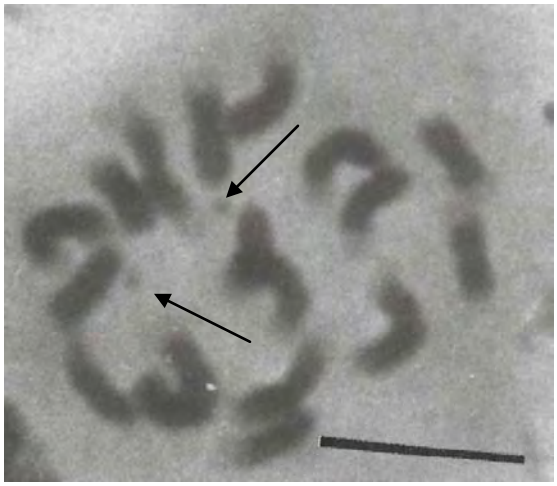
Figure 3 *Vernonia leopoldii* chromosomes. (A) Metaphase chromosomes, (B) Prometaphase chromosomes to show chromosome satellites (arrows), (C) Karyotype. Scale bars = 10 µm.

4.4 Vernonia galamensis (Cass.) Less.

The chromosome number of *Vernonia galamensis* is 2n = 18 (Table 3 and Figure 4A and B).The karyotype is composed of 16 median and 2 submedian chromosomes (Figure 4C). One pair of the chromosomes with satellites (secondary constriction) is observed in prometaphase chromosomes (Figure 4B). The chromosome size ranges from 3.4µm, the smallest to 5.9µm the largest. The total chromosome length is 79.4µm (Appendix 4).



A



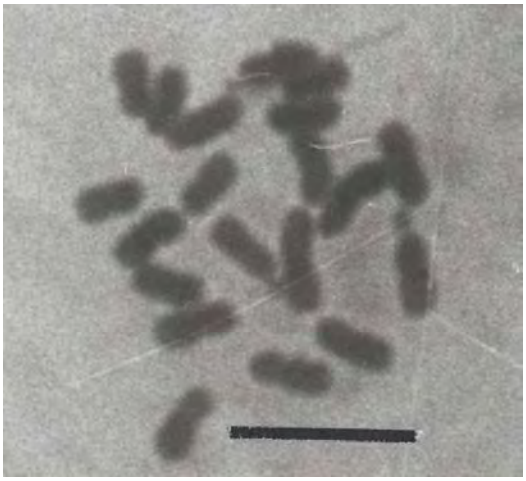
B

C

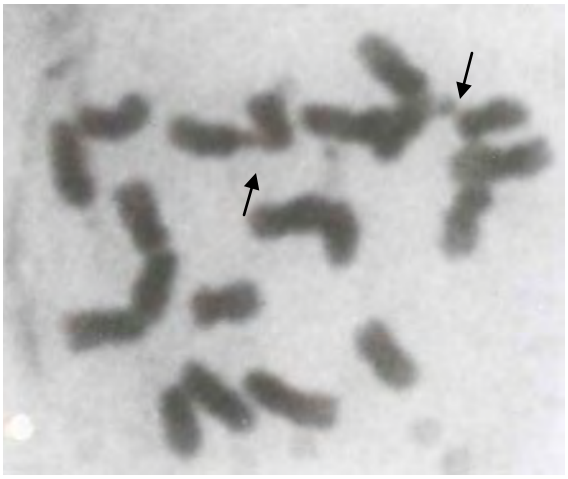
Figure 4 *Vernonia galamensis* Chromosomes. (A) Metaphase chromosomes, (B) Prometaphase chromosomes to show chromosome satellites (arrows), (C) Karyotype. Scale bars = 10 µm.

4.5 Vernonia schimperi DC.

The chromosome number of *Vernonia schimperi* is 2n = 20 (Table 3 and Figure 5A and B).The karyotype is composed of 20 median chromosomes (Figure 5C). One pairs of the chromosomes with satellites (secondary constriction) are observed in prometaphase chromosomes (Figure 5B). The total chromosome size is 80.8µm with individual chromosomes ranging from 2.9µm, the smallest to 5.9µm the largest (Appendix 5).



A



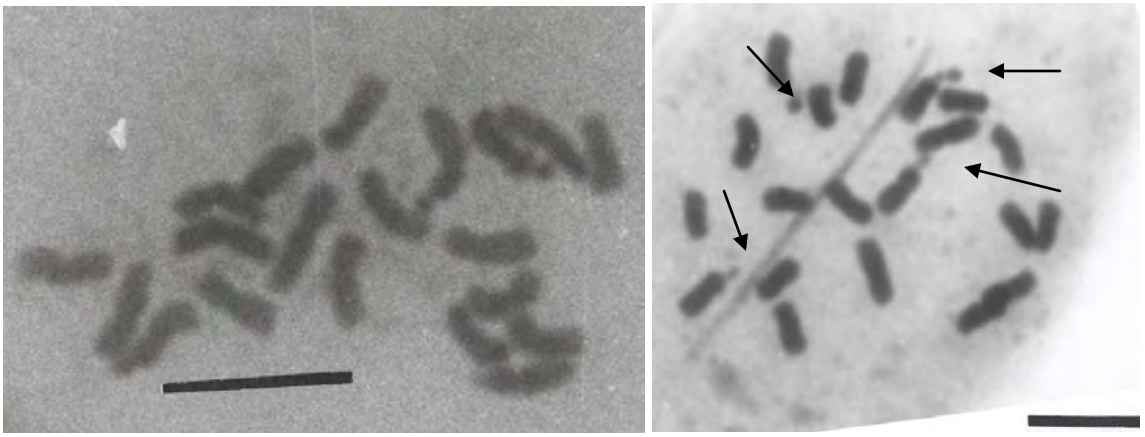
B

C

Figure 5 *Vernonia schimperi* chromosomes. (A) Metaphase chromosomes, (B) Prometaphase chromosomes to show chromosome satellites (arrows), (C) Karyotype. Scale bar = 10 µm.

4.6 Vernonia adoensis Sch. Bip. ex Walp.

The chromosome number of *Vernonia adoensis* is 2n = 20 (Table 3 and Figure 6A and B).The karyotype is composed of 20 median chromosomes (Figure 6C). Two pairs of the chromosomes with satellites (secondary constriction) are observed in prometaphase chromosomes (Figure 6B). The chromosome size ranges from 3.3µm, the smallest to 6.0µm the largest. The total chromosome length is 89.1µm (Appendix 6). The karyotype of *Vernonia adoensis* is symmetrical except for slight difference in size.



A B

C

Figure 6 *Vernonia adoensis* chromosomes. (A) Metaphase chromosomes, (B) Prometaphase chromosomes to show chromosome satellites (arrows), (C) Karyotype. Scale bars = 10 µm.

5 DISCUSSION

Most of previous works on chromosome of *Vernonia* are limited to reporting the number. Counts in the current study are in agreement with most of the previous reports on chromosome number of both specific species and the genus. Chromosome numbers of *V. adoensis*, *V. amygdalina* and *V. galamensis* were reported previously by Jones (1974), Adegbite and Ayodele (2004) and Thompson *et al.* (1994) respectively.

Chromosome number 2n = 20 was reported by Jones (1974) for *V. adoensis*, which agrees with the current count. Specimens for the report by Jones (1974) were collected from Uganda. Regardless of the geographic and latitudinal differences, the chromosome number remained the same for both Ethiopian and Ugandan *V. adoensis*. Chromosome number of 2n = 18 was reported for *V. galamensis* by Thompson *et al.* (1994) from plants grown in USA and 9 meiotic bivalents were reported from Ethiopian accessions by Getachew Ali (2008) which in other words mean 2n = 18. The result of the current study also confirms this number. Chromosome counts of 2n = 20 were found for *V. amygdalina* in this work, whereas it is reported as n = 20 (2n = 40) in the work of Adegbite and Ayodele (2004). The difference may arise from the existence of polyploidy series in the species. There is a wide difference in the locations of the specimens- the work of Adegbite and Ayodele was on specimens from Nigeria and the current study on specimens from Ethiopia, which differ in their longitudinal locations- which may result in difference in ploidy level of populations of the same species (Stebbins, 1971).

Chromosome counts of the other species (*V. leopoldii*, *V. myriantha* and *V. schimperi*) are reported here for the first time. However, the chromosome numbers of 2n =18 for *V. leopoldii* and 2n = 20 for *V. myriantha* and *V. schimperi* are in concordance with the previously suggested basic chromosome numbers for the genus *Vernonia* of x = 9 and x = 10 (Jones, 1974; Dematteis, 1998; Adegbite and Ayodele, 2004), respectively.

Most of the karyotypes of the species studied are symmetrical with most median, some submedian and one pair of subterminal chromosomes and slight difference in size of

chromosomes within karyotype. This karyotype symmetry is also in agreement with the work of Dematteis, (1998), on South American *Vernonia* species.

The number of satellited chromosomes that could be observed in the preparations is one pair in *V. galamensis*, *V. leopoldii*, *V. myriantha* and *V. schimperi* and two pairs in *V. adoensis* and *V. amygdalina*. All the secondary constrictions (satellites) were on the short arms of the chromosomes. There may be more chromosomes with secondary constriction or satellites but could not be detected due to different reasons such as very small size of the satellites, weakly stained satellites, satellites very far away from the other part of the chromosome and confuse with cell debris, chromosomes highly condensed and the constriction not visible (Stace, 2000).The determination of the exact number of secondary constriction and thus the numbers of satellites needs further study using techniques such as silver staining or *in situ* hybridization using rDNA probe.

The total chromosome length of a species is to some extent indicative of the genome size. However, it does not mean that the two are similar, because the chromosome length is affected by degree of condensation while the genome size is unaffected. The chromosome length of different species may vary due to difference in degree of condensation within limited range. However, it is possible to make comparison of genome sizes of different taxa based on the chromosome length, as both total chromosome length and genome size are results of DNA content. More precise estimate of genome size can be made by flow cytometry or densitometry.

On the basis of genome size the six *Vernonia* species studied in the present work can be arranged in to three groups as those having relatively small, medium and large genome sizes. Accordingly *V. galamensis* (2n = 18, chromosome length = 79.4 μm), V. schimperi (2n = 20 chromosome length = 80.8 μm), *V. leopoldii* (2n = 18, chromosome length = 82.1 μm) and *V. adoensis* (2n = 20, chromosome length = 89.0 μm) fall in the group with relatively small genome size. Particularly the first three species have almost similar genome size. *V. amygdalina* (2n = 20, chromosome length = 197.1 μm) has intermediate

genome size between the first four species and *V. myriantha* (2n = 20, chromosome length = 197.1 μm).

Although the species with 2n=20 tend to have larger genome size, there is no strong correlation between chromosome number and genome size. Among species with 2n = 20 the size range from 80.8 μm in *V. adoensis* to 197.1 μm in *V. myriantha*, which is more than two fold difference. Also *V. schimperi* with 2n = 20 has almost the same genome size with *V. galamensis* and *V. leopoldii* which have 2n = 18.

The genome size and chromosome number seem to have relationship with plant growth forms. The two species with chromosome number 2n = 18 and smaller total chromosome length are those with smaller physical height. *V. galamensis* is a herb and *V. leopoldii* is a woody shrub.

In constructing the karyotypes some chromosomes are difficult to locate centromeric position accurately. This may be because of the clarity of the chromosome images or degree of condensation. In some of the micrographs there is an overlap of chromosomes or parts of chromosomes, some of the chromosomes are bent and some are twisted so that the constricted region (centromere) could not be pointed. Some of the images lack good contrast with the background and blur the position of the centromere. This has posed difficulty in calculating arm ratio and expressing chromosome morphologies. This may have resulted in some degree of imprecision in arm ratio calculations.

6 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- ✓ Chromosome numbers of *Vernonia* species studied are n = 9 and n = 10, which agree with previous reports on the chromosome number of Old World *Vernonia* species. This supports the suggestion that Old World *Vernonia* have base number of x = 9 and x = 10 (Jones, 1974).
- ✓ From the karyotype morphologies and previous works that state the chromosome base numbers of x = 9 and x = 10, all the studied species are diploid. This supports the work of Dematteies (1998), that says the Old World *Vernonia* do not exhibit polyploidy.
- ✓ Though there is difference in size of the chromosomes, the karyotypes of the species studied are composed of symmetrical chromosomes with median and submedian centromeres.
- ✓ All chromosome satellites observed in the studied species are located on the shorter arm of the chromosomes.
- ✓ The genome size or chromosome number and the plants' growth forms are related in the genus. *Vernonia galamensis* which is an annual herb has the smallest genome size and chromosome number of 2n = 18. The other species with 2n = 18, *Vernonia leopoldii* is shrub.

6.2 Recommendations

- ➢ To provide fuller and more comprehensive view of the genus, the rest members of the genus are better studied cytogenetically.
- ➢ As use of more advanced cytogenetic techniques, such as *in situ* hybridization provide more detailed information both on the ploidy level and evolutionary history of the species, the chromosomes are better studied by using these techniques.

- These species are important, both economically and as natural biodiversity heritages and genetic wealth of the country and even the world, thus they are better recorded with their caryological data for their conservation, identification and sustainable use.

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APPENDICES

Appendix 1. *Vernonia amygdalina* chromosome measurements; arm lengths, arm ratio, total length of individual chromosomes, total length of all chromosomes and chromosome types*.

Chromosome	Length of short arm (in μm)	Length of long arm (in μm)	Total length (in μm)	Arm ratio	Chromosome type*
1	2.1	5.9	7.9	2.8	sm
2	2.0	5.8	7.8	2.9	sm
3	2.4	5.3	7.6	2.2	sm
4	2.4	5.2	7.5	2.2	sm
5	2.1	4.3	6.4	2.1	sm
6	1.9	4.1	6.0	2.2	sm
7	2.4	4.0	6.4	1.7	sm
8	2.2	4.0	6.2	1.8	sm
9	2.2	4.1	6.3	2.0	sm
10	2.1	3.9	6.1	1.9	sm
11	2.1	4.1	6.2	1.9	sm
12	1.8	4.2	5.9	2.4	sm
13	2.5	3.6	6.2	1.4	m
14	2.5	3.5	6.0	1.4	m
15	2.5	3.5	6.1	1.4	m
16	2.5	3.5	6.1	1.4	m
17	2.6	3.3	5.9	1.2	m
18	2.6	3.3	5.9	1.3	m
19	1.8	3.1	4.8	1.7	sm
20	1.6	2.9	4.6	1.8	sm
Total chromosome length			125.9		

* The chromosome types are based on nomenclature by Levan et al. (1964); M = median point, m = median region, sm = submedian region, st = subterminal region, t = terminal region and T = terminal point.

Appendix 2. *Vernonia myriantha* chromosome measurements; arm lengths, arm ratio, total length of individual chromosomes, total length of all chromosomes and chromosome types*.

Chromosome	Length of short arm (in μm)	Length of long arm (in μm)	Total length (in μm)	Arm ratio	Chromosome type*
1	4.2	7.7	11.9	1.8	sm
2	3.9	7.4	11.3	1.9	sm
3	2.9	7.6	10.6	2.6	sm
4	3.2	7.8	10.9	2.4	sm
5	3.8	6.5	10.2	1.7	sm
6	3.7	6.6	10.3	1.8	sm
7	3.8	6.2	10.0	1.6	m
8	3.9	6.4	10.2	1.6	m
9	2.4	7.9	10.2	3.4	st
10	2.4	8.0	10.4	3.4	st
11	3.1	7.1	10.2	2.3	sm
12	3.2	6.9	10.2	2.1	sm
13	4.1	6.2	10.3	1.5	m
14	4.0	5.9	9.9	1.5	m
15	3.2	5.6	9.2	1.7	sm
16	3.2	5.5	8.8	1.7	sm
17	2.5	4.8	7.2	1.9	sm
18	2.5	4.8	7.4	1.9	sm
19	1.8	5.1	6.8	2.9	sm
20	1.6	4.4	6.1	2.7	sm
Total chromosome length			192.1		

* The chromosome types are based on nomenclature by Levan et al. (1964); M = median point, m = median region, sm = submedian region, st = subterminal region, t = terminal region and T = terminal point.

Appendix 3. *Vernonia leopoldii* chromosome measurements; arm lengths, arm ratio, total length of individual chromosomes, total length of all chromosomes and chromosome types*.

No	Length of short arm (in μm)	Length of long arm (in μm)	Total length (in μm)	Arm ratio	Chromosome type*
1	2.9	3.6	6.6	1.2	m
2	2.6	3.5	6.1	1.4	m
3	2.2	2.8	5.1	1.3	m
4	2.1	2.6	4.8	1.2	m
5	1.9	2.6	4.5	1.4	m
6	1.8	2.6	4.5	1.4	m
7	2.1	2.4	4.5	1.1	m
8	2.2	2.4	4.6	1.1	m
9	1.9	2.5	4.4	1.3	m
10	1.9	2.6	4.5	1.3	m
11	1.8	2.7	4.5	1.5	m
12	1.8	2.6	4.4	1.5	m
13	2.1	2.1	4.2	1.0	M
14	2.0	2.1	4.1	1.0	M
15	2.0	2.1	4.1	1.1	m
16	1.9	2.1	4.0	1.1	m
17	1.6	2.1	3.7	1.2	m
18	1.7	2.0	3.7	1.2	m
Total chromosome length			82.1		

* The chromosome types are based on nomenclature by Levan et al. (1964); M = median point, m = median region, sm = submedian region, st = subterminal region, t = terminal region and T = terminal point.

Appendix 4. *Vernonia galamensis* chromosome measurements; arm lengths, arm ratio, total length of individual chromosomes, total length of all chromosomes and chromosome types*.

No	Length of short arm (in μm)	Length of long arm (in μm)	Total length (in μm)	Arm ratio	Chromosome type*
1	2.3	3.6	5.9	1.6	m
2	2.3	3.6	5.9	1.6	m
3	2.3	3.2	5.5	1.4	m
4	2.3	3.1	5.4	1.4	m
5	1.6	2.9	4.5	1.8	sm
6	1.6	2.7	4.3	1.7	sm
7	2.0	2.5	4.5	1.2	m
8	2.0	2.5	4.5	1.2	m
9	1.9	2.4	4.4	1.3	m
10	1.8	2.4	4.2	1.3	m
11	2.0	2.2	4.2	1.1	m
12	2.0	2.1	4.1	1.0	M
13	1.8	1.9	3.7	1.0	M
14	1.8	1.9	3.8	1.1	m
15	1.6	2.1	3.7	1.3	m
16	1.7	2.1	3.8	1.2	m
17	1.7	1.7	3.6	1.1	m
18	1.6	1.8	3.4	1.1	m
Total chromosome length			79.4		

* The chromosome types are based on nomenclature by Levan et al. (1964); M = median point, m = median region, sm = submedian region, st = subterminal region, t = terminal region and T = terminal point.

Appendix 5. *Vernonia schimperi* chromosome measurements; arm lengths, arm ratio, total length of individual chromosomes, total length of all chromosomes and chromosome types*.

Chromosome	Length of short arm (in μm)	Length of long arm (in μm)	Total length (in μm)	Arm ratio	Chromosome type*
1	2.6	3.2	5.9	1.2	m
2	2.5	3.1	5.6	1.3	m
3	2.2	2.4	4.6	1.1	m
4	2.1	2.6	4.6	1.3	m
5	2.1	2.5	4.6	1.2	m
6	2.0	2.6	4.6	1.3	m
7	2.0	2.5	4.5	1.2	m
8	1.9	2.5	4.4	1.3	m
9	1.9	2.2	4.2	1.2	m
10	2.0	2.2	4.2	1.1	m
11	1.9	2.0	3.9	1.1	m
12	1.8	2.0	3.8	1.1	m
13	1.8	2.1	3.8	1.2	m
14	1.8	2.1	3.9	1.1	m
15	1.5	1.8	3.2	1.0	M
16	1.5	1.6	3.1	1.1	m
17	1.5	1.5	3.0	1.0	M
18	1.5	1.5	3.0	1.0	M
19	1.5	1.5	3.0	1.0	M
20	1.4	1.5	2.9	1.1	m
Total chromosome length			80.8		

* The chromosome types are based on nomenclature by Levan et al. (1964); M = median point, m = median region, sm = submedian region, st = subterminal region, t = terminal region and T = terminal point.

Appendix 6. *Vernonia adoensis* chromosome measurements; arm lengths, arm ratio, total length of individual chromosomes, total length of all chromosomes and chromosome types*.

Chromosome	Length of short arm (in μm)	Length of long arm (in μm)	Total length (in μm)	Arm ratio	Chromosome type*
1	2.4	3.6	6.0	1.6	m
2	2.2	3.6	5.8	1.6	m
3	2.1	2.9	5.0	1.4	m
4	2.4	2.9	5.3	1.2	m
5	2.0	2.5	4.5	1.2	m
6	2.2	2.5	4.8	1.1	m
7	2.1	2.2	4.4	1.0	M
8	2.0	2.1	4.2	1.0	M
9	2.0	2.4	4.5	1.2	m
10	2.1	2.4	4.5	1.1	m
11	2.0	2.2	4.2	1.0	M
12	2.1	2.2	4.3	1.0	M
13	2.1	2.2	4.4	1.0	M
14	2.1	2.2	4.3	1.0	M
15	2.0	2.2	4.2	1.1	m
16	2.1	2.2	4.4	1.0	M
17	1.6	2.1	3.8	1.3	m
18	1.7	2.0	3.8	1.2	m
19	1.5	2.0	3.5	1.3	m
20	1.4	1.9	3.3	1.3	m
Total chromosome length			89.0		

* The chromosome types are based on nomenclature by Levan et al. (1964); M = median point, m = median region, sm = submedian region, st = subterminal region, t = terminal region and T = terminal point.

Declaration

I, the undersigned, declare that this thesis is my original work, it has never been submitted in any institution for other degree or for publication and that all sources of materials used for the thesis have been duly acknowledged.

Name_____

Signature_____

Date of submission_____

Approved by:

Name_____

Signature_____

Date _____