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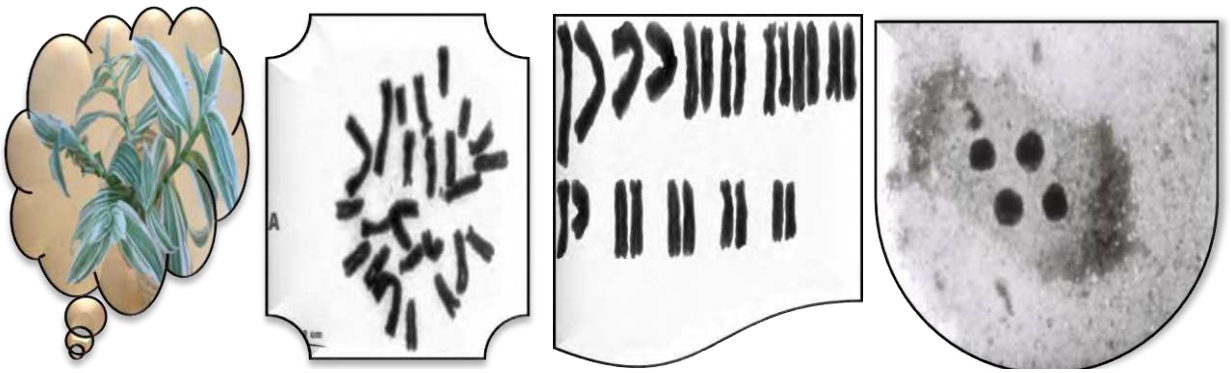

Chromosome Study of Some Species of *Commelina* L. and *Tradescantia* L. (Commelinaceae) From Ethiopia

By

Samuel Gebrekiristos

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)

March 2012

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Abbreviations

A.A	Addis Ababa
A_1	Intra-chromosomal asymmetry index
A_2	Inter-chromosomal asymmetry index
C-banding	Constitutive heterochromatin banding
DI	Dispersion index
FISH	Fluorescent *in situ* hybridization
G-banding	Giemsa banding
Kpb	Kilo base pair
Mbp	Mega base pair
n	Gametic chromosome number
pg	Pico gram
NORs	Nucleolar organizer regions
R-banding	Reverse banding
r DNA	Ribosomal DNA
T-banding	Telomeric banding
x	Basic chromosome number

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Abstract

Karyotypes and nucleolar number of four species of the genus *Commelina L.* and two species of the genus *Tradescantia L.* (Commelinaceae) which were collected from different localities of central and south-western part of Ethiopia (Addis Ababa, Ginchi, Sebeta, Entoto and Jimma), were analysed. Somatic chromosomes were prepared from root tips that emerged from the nodes of stem cuttings that were made to stand submerged in water. The roots were pretreated in 8-hydroxyquinelin 3-5 hrs followed by fixation in 3:1 ethanol: acetic acid for 1-24 hrs at 4^{O}C. Air-dry slides were prepared following cellulase and pectinase maceration at 37^{O}C, the preparation was stained in Giemsa stain (PH 6.4), rinsed and mounted. Nucleoli were stained in silver nitrate solution. Chromosome numbers and Karyotype formula of the six species were found as *C. africana* (A.A) 2*n*=2*x*=30 (12m + 10sm + 8st), *C. africana* (Sebeta) 2*n*=2*x*=30 (14m + 10sm + 6st), *C. benghalensis* 2*n*=6*x*=66 (36m + 24sm + 6st), *C. diffusa* (Entoto) 2*n*=2*x*=30 (16m + 6sm + 8st), *C. diffusa* (Ginchi) 2*n*=66 (28m +26sm + 12st), *C. diffusa (*Jimma*)* 2*n*=2*x*=30 (10m + 8sm + 12st), *C. subulata* 2*n*=2*x*=30 (18m +10sm + 2st), *T. zebrina* 2*n*=2*x*=24 (4m + 6st + 14t), *T. fluminensis* (green) 2*n*=60 (8m + 6sm + 22st + 24t) and *T. fluminensis* (variegated) 2*n*=60. According to Stebbins karyotype asymmetry, the karyotypes of *C.africana* (A.A), *C. diffusa* (Entoto), *C. subulata* were 2A type, while that of *C. africana* (Sebeta), *C. benghalensis* and *C. diffusa* (Ginchi) were 2B type. 3A asymmetry type was obtained for *C. diffusa* (Jimma) and 3B asymmetry type was also found for *T. zebrina* and *T. fluminensis* (green). Karotypes of *Tradescantia* were found to be bi-modal, while it is monomodal for the *Commelina* species of the studied plant materials. Satellites were observed for species *C. africana*, *C. diffusa* and *T. zebrina* with variation in number ranging from 2 to 6. The maxiumum number of nucleoli observed varies from two to four for *Commelina*, while, it is three for *Tradescantia*. This study reported karyotype and nucleoli of the Ethiopian Commelinaceae for the first time. The current investigation can be considered as an additional karyotype data to the earlier meiosis report for Ethiopian materials.

Key words: *Commelina L.*, Ethiopia, Karyotype, Nucleoli, Satellite, *Tradescantia L.*,

1. Introduction

The present study has mainly focused on four species of *Commelina* and two species of *Tradescantia*. Taxonomically the two genera were classified under family Commelinaceae, order Commelinales and class Liliopsida (Faden, 1998). *Tradescantia* is also known to be included in sub-tribe Tradescantiinae and tribe Tradescantieae (Faden, 1998).

The family Commelinaceae, commonly known as the spiderwort, is known to possess 41 genera and 650 species throughout the world (Kubitzia, 1998), with main distribution in tropics and subtropics that extends into northern temperate regions. The family is represented by 9 genera and 56 species in Ethiopia and Eritrea with four of the species of *Tradescantia* known in cultivation (Ensermu Kelbessa and Faden, 1997).

The genus *Commelina* is a perennial or annual herb with fibrous or tuberous roots. It has creeping, ascending or erect, and branched stems with often cleistogamous flower-bearing underground stolons. The name *Commelina* was derived after the Dutch botanists Jan Commelijn (1629-1692) and his nephew Caspar (1667/1668-1731) with about 170 or more species occurring in the warmer countries of the world (Mudua, 2007). This genus contains about 250 species and is the largest in number within the family Commelinaceae (Morton, 1967).

With about 100 species, *Commelina* is the largest genus of Commelinaceae in Africa. At least 65 species occur in the combined areas of the Flora of Tropical East Africa (Kenya, Uganda, and Tanzania) and Flora Zambesiaca (Malawi, Mozambique, Zambia, Zimbabwe, and Botswana) (Faden, 1994; Faden and Alford, 2001). About 19 species have been identified in Ethiopia and Eritrea including 2 undescribed species that are endemic to Ethiopia (Ensermu Kelbessa and Faden, 1997).

Although several medicinal and economic uses of the family Commelinaceae are known, little has been done in cytological analysis in Ethiopia. Previous research done by Lewis and Taddesse Eba (1964) only focused on chromosome number of four species included in this study based on meiosis and voucher specimen were taken only from the Harar Province except for *Cyanotis barbata* D. Don which was from Debrezeit. The present study was focused on chromosome number, karyotype analysis and silver staining.

The genus *Tradescantia* is a perennial herb which contains branched, creeping, erect or trailing stems rooting at lower nodes (Mabberley, 1997; Ensermu Kelbessa and Faden, 1997). Inflorescence is located either terminally or axillary and petals are three in number with blue, rose, purple or white colour. The flowers have few to numerous in number and also pedicellate. About 60 species included in the genus *Tradescantia* have been identified in North, Central, South America and West India with several taxa under cultivation (Ensermu Kelbessa and Faden, 1997). The chromosome size was mostly large with rare cases of either medium sized or small and basic chromosome number ranges from 4 – 13 (Faden and Hunt, 1991).

This thesis work is intended to provide cytological data on some of the species of Commelinaceae, namely *C. africana*, *C. benghalensis*, *C. subulata*, *C. diffusa*, *T. fluminensis* and *T. zebrina*. Here the chromosome number and ploidy level of these species of Commelinaceae are described. Such study can help to expand the current cytological knowledge of the Ethiopian Commelinaceae with further contribution for phylogenetic and biosystematics research.

Generally, it is hypothesized that basic chromosome number of *Commelina* is 15, karyotype formula is the same within a species but different across species of the genera and polyploidy observed within a species can be associated with variation in morphological character.

2. Literature Review

2.1 Taxonomic description of Commelinaceae

Members of this family are characterized by perennial or occasionally annual habit plants possessing fibrous or tuberous roots, terminal or auxiliary inflorescence and closed sheath (Fadden, 1998). The earlier classification of Commelinaceae was based on floral features and this has been modified through incorporating other important taxonomic characters like anatomical, palynological and cytological data (Faden and Hunt, 1991).

Based on anatomical and palynological attributes, the family Commelinaceae is further classified into two subfamilies, Cartonematoideae and Commelinoideae . The subfamily commelinoideae has distinct features that differentiates from the former due to the presence of raphide canals and grandular hairs with various flower colors mostly pink or blue to white and rearly yellow to orange (Faden and Hunt, 1991). Commelinoideae is further divided into two tribes, Tradescantieae and Commelineae. Observation of moniliform stamen hairs with chromosome size mostly medium to large makes Tradescantieae unique from the other tribes (Faden and Hunt, 1991). This is true for *Tradescantia*, *Tinantia*, *Cochliostema*, *Cyanotis*, *Palisota* and *Streptolirion* (Faden and Hunt, 1991). Chromosome size of Commelineae was found to be mostly small (Jones and Joppings, 1972), with some exceptions found, for instance, in *Streptolirio* which possessed large sized chromosomes (Faden and Suda, 1980).

Both the genera *Tradescantia* and *Commelina* possess inflorescence enclosed in or subtended by spathes. *Commelina* is characterized by clustered spathes, symmetrical flowers with 3 stamens, 2-3 staminoids and glaborous filaments. Species's found within *Tradescantia*, on the other hand,

possess mostly paired spathes, regular flowers, 6 stamens with beared filaments on the lower half (Ensermu Kelbessa and Faden, 1997).

2.2 Geographic distribution

The main centers of diversity for Commelinaceae include Mexico (especially Oaxaca and Chiapas), north Central America (in case of sub tribe Tradescantiinae and Thysantheminae), tropical Africa (including Madagascar), Thailand and southwestern China (especially for Commelinae, Cynotinae and Streptoliriinae). The greatest diversity has also been recorded in Africa with nearly half of the genera and 40% of the species are found (Faden, 1983). The family is ecologically diverse which mainly grows in humid and messy habitats along with forests and grasslands with less abundance in dry area (Kubitzia, 1998).

Generally, the species found within Commelinaceae grows throughout tropical and temperate regions with few known wide spread, weeds occurring throughout the globe (Ensermu Kelbessa and Faden, 1997).

2.3 Botanical description of the study species

The present study includes four species from the genus *Commelina* and two species from the genus *Tradescantia*: including *C. benghalensis, C. africana, C. diffusa, C. subulata, T. fluminensis* and *T. zebrina*. The botanical descriptions of these species are briefly presented below.

2.3.1 Commelina benghalensis L.

Commelina benghalensis has an erect or creeping stems with an ascending position of 0.9-2.5 m long that branch and root at the nodes (Ensermu Kelbessa and Faden, 1997). Leaves are ovate or elliptical with a base narrowed into a petiole. Flowers have three lilac blue petals with the lower

smaller than the two laterals and they are occasionally white. The fruit consists of a pear-shaped capsule with five seeds and the capsule open when mature (dehiscent). Seeds are ribbed-rough, greyish brown and sometimes appear sugar-coated. The species can be typically identified by its blue flowers, short flower stalk with no extension above the spathe, partial joining of spathe margins and reddish brown hairs of the leaf sheath (Ivens, 1967).

Commelina benghalensis occurs in altitudes ranging from 400 to 2500 *m.a.s.l* and grows in forest, woodland, stream banks, waste grounds, rocky hillsides, gardens and under bushes with partial shades. *Commelina benghalensis* is widely recognized as a weed of coffee, cotton, groundnut, pepper and teff (Ensermu Kelbessa and Faden, 1997). This is originally an Old World species and then introduced to Americas and Hawaii. It grows in Madagascar, Mascarene Islands and throughout tropical Africa from Cape Verde and Senegal to Ethiopia, and south to South Africa (Van der Burg, 2004b). In Ethiopia, it is distributed in most floristic regions of the country including Welo, Gojam, Shewa, Welega, Iubabur, Kefa, Gomo Gofa, Sidamo and Harerge (Ensermu Kelbessa and Faden, 1997).

2.3.2 Commelina africana L.

Commelina africana is a perennial herb which roots at nodes and has medium size flowers (Morton, 1956). The plant is up to 0.5 m long, glabrous pubescent with hard, thick and long roots. Leaves can be flat or folded with diversified shape, ranging from linear to rectangular and smaller, glabrescent to variously hairy. The species has generally well developed inflorescence containing two cymes (flower clusters) and yellow petals (Mudau, 2007). Seeds vary in outline from cylindrical to rectangular with variation in size (Ensermu Kelbessa and Faden, 1997).

Commelina africana is an indigenous and widespread herb of Africa, occurring from Senegal to Ethiopia, and up to South Africa. It also grows in Saudi Arabia, Yemen and Australia (Van der

Burg, 2004a). The species is typically identified by its yellow instead of blue, purplish or pink flowers (Van der Burg, 2004a).

2.3.3 Commelina diffusa Burm. f.

Commelina diffusa is a diversified annual or perennial herb of the tropics with a loose leaf sheath and frequently mottled dark red markings. Stems creeping with rooting at lower nodes, erect over 15-60 cm and glabrous or pubescent in a row corresponding to the fused edge of the leaf sheath beyond the internodes. First flowers are male that are born on long pedicels, while others are hermaphrodite plus shortly pedicellate (Morton, 1956). According to Morton (1967) the species broadly classified into subsp. *diffusa,* subsp. *aquatic* Morton and subsp. *montana* Morton.

The flowers are arranged into two cincinni or scorpioid cymes. The lower cincinnus bear 2 to 4 bisexual flowers on a shorter peduncle, while the upper cincinnus has one to several male flowers on longer peduncle. The membranous sepals are unremarkable at only 3 to 4 mm length. Petals are predominantly blue or rarely violet or white. The flowering season range from May to November (Hong *et al.*, 2000).

The species is mostly confined to pan-tropical and subtropical moist places by streams, in riverine woodland and forest, among grasses and sedges beside paths and as a weed in cultivated fields at altitudes ranging from 500-2400 m. In Ethiopia it grows located in different floristic regions including Gonder, Gojam, Shewa, Welega, Ilubabor, Kefa, Gamo Gofa, Sidamo, Bale and Harerge (Ensermu Kelbessa and Faden, 1997).

2.3.4 Commelina subulata Roth

Commelina subulata is an annual plant with fibrous to fusiform roots. Stems are either erect or creeping at base which gradually ascends above with open leaf sheath or rarely closed (Ensermu

Kelbessa and Faden, 1997). The species is glabrous containing falcate spathes that are fused at a base with small rectangular seeds 1 to 1.5 mm long (Morton, 1967). It is widely recognized as weed of wheat, teff and maize which mainly occurs in different countries of Africa and Asia. The species is also commonly distributed in northern, central, south and southeastern parts of Ethiopia (Ensermu Kelbessa and Faden, 1997).

2.3.5 Tradescantia zebrina Hort. ex Bosse

Tradescantia zebrina is a low spreading succulent herb with purple and silvery-green leaf blades plus hairy petiole sheathing the stem. The species has pollen displaying two germinal or pseudo-germinal apertures on the proximal face of the grain, a unique feature to the tribe, but similar in all other aspects to that of *Tradescantia* (Poole and Hunt, 1980).

Tradescantia zebrina is generally a rhizomatous, perennial, stem creeping and rooting at lower nodes below and ascending above, glabrous and internodes of 6-9 cm long. The leaf-sheaths have long, purple-veins and hairy in nature. The species is native to West Indies, Central and South America, but now, cultivated in several tropical and sub-tropical countries including Ethiopia, where it is grown in Shewa and Harerge floristic regions in altitudes ranging from 1200 to 2500 *m.a.s.l* (Ensermu Kelbessa and Faden, 1997).

2.3.6 Tradescantia fluminensis Vell.

This species has *Tradescantia albiflora* as a synonym. It is generally a procumbent succulent herb with stems creeping and rooting at the lower nodes below, ascending above, glabrous except for the uppermost internodes. Leaf sheaths are normally closed. Leaves are nearly sessile, wider near the base and green with white variegation longitudinally. The inflorescences can be sub-terminal or falsely terminal. Although *T. fluminensis* is native of Brazil and Paraguay, it is now cultivated in several countries including Ethiopia (Ensermu Kelbessa and Faden, 1997).

2.4 Uses of the study plant

A number of species of the genus *Commelina* and *Tradescantia* are known to have various uses such as medicinal, food, feed and ornamental.

2.4.1 Folk medicine

The genus *Commelina* has several medicinal significances. *C. benghalensis* was in use to treat bed sores, breast sores and pimples in Pakistan (Qureshi *et.al,* 2008). In East Africa, the sap of *C. benghalensis* leaves and stems is used to treat ophthalmia, infertility in women, leprosy, sore throat and burns, and the liquid contained in the flowering spathe is used to treat eye complaints in Zanzibar (Van der Burg, 2004b). People from Nepal use a paste derived from the plant to treat burns, and indigestion with a juice produced from the roots (Manandhar *et al.*, 2000). In China, *C. diffusa* is used as a medicinal herb with febrifugal and diuretic effects (Hong *et al.,* 2000).

The ash of *C. africana* is used as one of the ingredients in a Sotho charm application to the loins for sterility and an infusion is drunk for the same purpose (Mudau, 2007*).* In Kenya, an infusion of the plant is used as a wash to reduce fever, and pounded stalks are used to treat colds and coughs in children. Fluid from the spathes is applied locally to cure eye diseases. The Zulu of South Africa bathe the body, especially of a child, with a cold infusion in cases of restless sleeping. The Sotho in Southern Africa takes a decoction of the plant with *Tephrosia capensis* Pers. for treatment of a 'weak heart' and nervousness. In Democratic Republic of Congo the root is used for the same purpose. The plant cooked with *Haplocarpha scaposa* Harv., *Helichrysum pilosellum* (L.f.) Less. or the root of *Cotyledon decussata* Sims is given by Sotho as medicine to young women to cure infertility. Also, an infusion of the plant is drunk and its ash is rubbed over the loins as a fertility charm. In Zimbabwe and South Africa, a concoction of the root is used as

treatment for veneral diseases and to treat women with menstrual cramps. This preparation is also used for pelvic pains (Van der Burg, 2004a).

2.4.2 Modern medicine (pharmaceuticals)

In the conventional pharmaceuticals, drug discovery has recently shifted from synthetic models and compounds to natural organisms including plants and animals as this is not fully exploited (Jemilat *et al.,* 2010).

Numerous compounds have been identified from the vegetative and flower parts of *Commelina benghalensis* including noctacosanol, n-triocotanol, stigma-sterol, compesterol and hydrocyanic acid (Jayvir *et al*, 2002). Phyto-chemical screening also revealed the presence of many secondary metabolites like phlobatannins, carbohydrates, tannins, glycosides, volatile oils, resins, balsams, flavonoids and saponins (Jemilat *et al.,* 2010). Presence of flavonoids, for example, indicates the plant might have an antioxidant, anti-allergic, anti-inflammatory, anti-microbial or anti- cancer activity (Kunle and Egharevba, 2009). In addition, *C. benghalensis* has analgestic action that proves the folkloric use in pain management (Hasan *et al.,* 2010).

2.4.3 Ornamental and other values

Commelinaceae are the popular houseplants grown in different countries of the world. Both *Tradescantia zebrina and Tradescantia fluminensis* are widely cultivated in home gardens for aesthetic purpose (Glimn-Lacy and Kaufman, 2006). Plants like *C. africana* are exploited for decoration (Mudau, 2007*). D*yes produced from the juice of the petals of *C. diffusa* are widely used for painting in China (Hong *et.al,* 2000).

2.4.4 Food and fodder

During famine period, peoples of India and Philippines use *C. benghalensis* as source of food (NAPPO, 2003).

Although seldom cultivated, *Commelina* spp. and other tropical native forages often form part of unimproved pastures in tropics and therefore play a critical role in providing supplementary crude protein (CP) to grazing and browsing ruminants. As such, they are important assets in small farm systems where, owing to their abundance, they are often harvested for feeding of stall-fed ruminants. Various Commelinaceae species have been reported to have roles as ruminant feeds on smallholder farms (Geesing and Djibo, 2001). In Kenya and Tanzania, the leaves are fed to livestock, especially pigs and rabbits. The flowers can also be used as source of bee forage (Van der Burg, 2004a).

2.5 Cytogenetics of Commelinaceae

Until 1980's chromosome counts for 49 genera and approximately 37 % species found within the family Commelinaceae has been published (Faden and Suda, 1980). Their chromosome size ranges from small to apparently large (Faden and Suda, 1980). Basic chromosome number of Commelinaceae ranges from smallest x=4 (*Gibasis*) upto x=29 (in *Rhopalephora*) (Faden, 1998). Some genera with apparently small number of species like *Stanfieldiella* (x=11), *Polyspatha* (x=14) and *Buforrestica* (x=17) possess single basic chromosome number, whereas most of the genera of the family including *Aneilema* (x=9, 10, 13-16), *Commelina* (x=11-15) and *Murdannia* (x=6, 9-11) contain multiple basic chromosome numbers (Faden, 1998). In addition, bimodal character of chromosomes is also common for some genera like *Anthericopsis*, *Floscopa*, *Tinantia* and occasionally in species within other genera (Faden, 1998).

Polyploidy and aneuploidy seem to be frequent in Commelinaceae (Morton, 1967; Faden and Suda, 1980). Aneuploidy series of four or more base numbers occur in *Aneilema*, *Commelina*, *Cyanotis*, *Cymbispatha* and *Murdannia* (Faden and Suda, 1980).

2.5.1 Commelina

According to Jones and Joppings (1972), the chromosome size of *Commelina* was generalized as small. However, Faden and Suda (1980) found medium to relatively large sized chromosomes based on their studies on African and Asian materials. Morphologically, chromosomes of *Commelina* are largely metacentric and sub-metacentric with the exception of those of *C. macrosperma* and *C. zambesica* which vary greatly (Faden and Suda, 1980).

The chromosome number of half of the species of *Commelina* has been reported with 40 different numbers ranging from $2n = 16$ (Zheng *et al*., 1989) to $2n$=180 (Jones and Jopling, 1972). One third of the recorded species show more than one chromosome number with the same or different ploidy levels, 75% of which have cytotypes that are not multiples of a single basic chromosome number. Most of the recorded species present at least one diploid cytotype, and $2n = 30$ (27%) and $2n = 28$ (19%) are the most common (Jones and Jopling, 1972). Several basic chromosome numbers have been proposed for *Commelina*, $x = 7$ and 11 to15 (Jones and Jopling, 1972), of which the most common are $x = 15$ and 14. Polyploid components of a species are associated with different habitat from that of diploid. In *C. diffusa* and *C. benghalensis,* the polyploids are associated with hilly or mountainous habitats of higher rainfall (Morton, 1967).

Cytogenetically, study from different localities of Japan, India, Pakistan, Taiwan and Southeast Asia of the species *C. benghalensis* point out the constancy of diploidy of $2n$=22 (Sharma, 1955; Malik, 1961; Alam and Sharma, 1981; Bhattacharia, 1975; Fujishima, 2007a). However, chromosome numbers and ploidy levels vary for African populations of the species $2n$= 22, 44,

56 and 66 (Lewis and Taddesse Eba, 1964; Morton, 1956, 1967). In Ethiopia, both diploid and tetraploid races had been reported (Lewis and Taddesse Eba, 1964). *C. benghalensis* from materials of California were also approximately hexaploid of 2*n*=66 (Faden, 2007). Shingebu and Kobori (1997) pointed out the diversity of chromosome number and satellite size that are associated with invasion of the species to new habitat. On the other hand, Fujishima (2007a) observed variation in chromosome satellite among the population, within particular population and even plants grown under similar ecological environment revealing the negative association between satellite chromosomal complement and ecological environment.

Commelina diffusa is a cosmopolitan species with diverse chromosome numbers 2*n* = 28, 30, 56, 60, 72, 86, 90, 120 (Sharma, 1955; Morton, 1956, 1967; Lewis, 1964; Briggs, 1966; Jones and Jopling, 1972; Bhattacharya, 1975; Faden and Suda, 1980; Fujishima, 2007b), but 2*n* = 2*x* = 30 is the most common. Alam and Sharma (1984) pointed out that 2*n*= 30 karyotypes of *C.diffusa* from different population in India possess variation among themselves. Plants from Japan with 2*n*=72 (24m+38sm+10st) composed of three chromosomal groups namely m, sm and st and their karyotype slightly differed within each other (Fujishima, 2007b). The 2*n*=60 (18m+36sm+6st) is also counted in a plant from Japan and Australian species possess, 2*n*=82 with bimodal karyotype (Fujishima, 2007b).

Earlier chromosome counts were obtained for *Commelina africana* in different countries within African sub-continent; 2*n*= 30 from Ethiopia and Sierra Leone (Lewis and Taddese Eba, 1964; Morton, 1967) and 2*n*= 28 in Ghana (Morton, 1956). But in South Africa both 2*n*= 60 and 120 were counted (Lewis, 1964). In West Africa, the forest populations have a diploid number of 28, while the savanna populations produce 30 chromosome counts (Morton, 1967).

2.5.2 *Tradescantia*

Four chromosome numbers having diversified karyotype, from the standpoint of primary constrictions and chromosome size, with three in aneuploid series were known in Tradescantineae. Cytological study indicated that the genus *Tradescantia* contains at least four basic numbers and five karyotypes (Celarier, 1955). Morphologically, the chromosomes of the United Stated species of *Tradescantia* are found to be large, all similar in size, and with either median or sub-median primary constriction (Celarier, 1955).

Tradescantia zebrina has 2*n*= 23 and 2*n*= 24 chromosome numbers (Zhang, 1989; Sakurai and Ichikawa, 2001). According to Darlington (1929), the 24 chromosomes are grouped into 4 median, 8 sub-terminal and 12 terminal chromosomal types. The karyotype formula based on Sakurai and Ichikawa (2001) is 2*n* = 4 M + 6 ST + 14 T for both *Zebrina pendula* and *Zebrina purpusii*, whereas *Z. pendula* cv Quadricolor has 2*n*=6m + 5 st + 11 T+ 1 SA. But the Mexican plants of *Z. pendula* have common karyotype 2*n*= 4M+ 8A+12T, where the 2*n*= 23 chromosome plant possess 5M+ 8A+ 10T (White, 1945; cited in Shingebu *et al.,* 2002). The longest sub-telocentric chromosome contains satellites at the short arm in *Z. pendula* and at longer arm in *Z. purpusii*. In *Z. pendula* cv Quadricolor, conversely, only the unique short acro-centric chromosome pair have satellites on the long arm, none of the five sub-telocentric chromosomes possess satellites (Sakurai and Ichikawa, 2001).

Both the green and variegated forms of *T. fluminensis* have 30 haploid chromosomes. Among the 2*n*=60, 44 chromosomes have sub terminal constriction, while the rest 6 are long with terminal constriction (Darlington, 1929).

2.6 Implication of cytological study in karyosystematics

During the past few decades a declining trend of cytogenetics research, especially on numerical and structural change of chromosomes, had been observed. The increased interst in construction of molecular maps and genome sequencing, however, led to rebirth of cytogenetics (Gupta, 2006). The analysis of chromosomal structure further enabled the identification of chromatin content including nucleosome and chromatosome subunits, which perform several level of folding for the purpose of efficient packaging within chromosome boundaries (Gupta, 2006).

The efficient utilization of karyological data in taxonomy, traditionally referred to as cytotaxonomy or karyosystematics (Greilhuber and Ehrendorfer, 1988) and this contributes to evaluate the genetic relationship among species or populations and to a better understanding of the way they diverged from each other (Guerra, 2008).

Chromosome number is the simplest karyotype parameter that gets special attraction by cytotaxonomists. It is the quickest, cheapest, and easiest way to get any substantial information about the genome of a species. The chromosome number is a well known cytotaxonomic datum for almost all families and most plant genera. A lot of other karyological information can be added to this initial karyotype description, as chromosome size and morphology, karyotype symmetry, banding patterns, and position of satellite DNAs on chromosome (Greilhuber, 1995).

There is a need for development of additional techniques for efficient resolution and karyotyping in cytogenetics. The crucial data of plant systematics and evolution range from chromosome number to details of molecular cytogenetics. In the past, plant chromosome number is the sole for almost every kind of cytogenetic research. But nowadays, with the development of cytomolecular techniques, mainly FISH (Fluorescent *In Situ* Hybridization) with its various categories, plant cytogenetics research has greatly advanced, revealing unexpected details of

chromosome behaviour and evolution (Guerra, 2005). FISH identifies different landmarks for physical chromosome mapping and fine karyotype comparison among related species (Kato *et al.,* 2004).

2.7. Karyotype

Karyotype can be described as the number and phenotypic appearance of chromosomes of particular species (Jackson, 1971). The karyotype is an end result of many forces acting on the genome at structural, organizational and functional levels that attempt to couple with mitosis, meiosis and the diversified functional states of the interphase cells during the entire life of the organism. Being a genetic material and showing a full map of the genome organization, karyotypes differ from other phenotypic characters like floral morphology or isozyme bands (Guerra, 2008).

Differences in karyotype are essential for making many taxonomic decisions and telling clues in unraveling evolution, for instance, in tracing the parentage of hybrids or the origin of genomes in polyploids (Stace, 2000).

2.8. Constituents of karyotype

Karyotype of particular species, genera or families can greatly differ from one another to various degrees and the numbers of demonstrable ways by which they differ are constantly increasing with the development of new techniques. This variation can be associated with spontaneous genomic changes leading to additional visible chromatins which can be detrimental to varying degrees at the phenotypic level.

Karyotype can be classified as symmetrical and asymmetrical based on measurement of arm ratio and chromosome length. Symmetrical karyotypes predominantly possess metacentric or

sub- metacentric chromosome with roughly same size (Arabbeigi *et al.,* 2011). While karyotypes consisting of unequal chromosome lengths with sub-median or sub-terminal centromere are termed as asymmetric karyotypes. Inorder to measure the degree of karyotype asymmetry, Stebbins (1971) quantify four ratios of smallest to longest chromosome within a complement (1, 2, 3, 4) with its proportion of metacentric chromosomes (A, B, C, D) so that sixteen classes of karyotypes are able to produce in increasing order of asymmetry (1A, 1B, 1C, 1D....., 4A, 4B, 4C, 4D). Increasing asymmetry can take place either by shift in centromere position from median/sub-median to terminal or sub-terminal positions or accumulation of dissimilarity in the relative size between the chromosomes of the complement (Arabbeigi *et al.,* 2011). The species with more symmetric karyotypes and higher number of chromosomes are considered more primitive in a certain group of plants (Stebbins, 1971). But in some cases symmetrical can be derived from asymmetrical karyotype through Robertsonian fusion as in rodents (Mao *et al.,* 2008).

A description of the karyotype of a species includes the following characteristic features: (1) the chromosome number, (2) the total length of the chromosome complement (genome size), (3) size and location of the centromere (4) the absolute and relative sizes of chromosomes, (5) position, number, size, and distribution of differentially staining heterochromatic segments, (6) number, size, and position of secondary constrictions and satellites, (7) and the degree of symmetry or asymmetry (Jackson, 1971; Levin, 2002). These components of karyotype will be briefly discussed bellow, followed by discussion of mechanisms by which chromosome polymorphisms arise and subsequently lead to evolution of karyotypes.

2.8.1 Chromosome number

Chromosome numbers of eukaryotes is highly diversified with lowest number known in ant, *Myrmecia croslandi* Taylor (2*n*=2) (Crosland and Crozier, 1986). In angiosperms, however, the lowest chromosome number has been reported by Bennet *et al.,* (1986) in *Zingeria biebersteiniana* (Claus) (2*n*=4). The higher record in number of chromosomes is so far held by the fern *Ophioglossum reticulatum,* with 2*n* = 1440. Likewise, in dicots the highest number is 2*n*= 640 for *Sedum suaveolens* (Crassulaceae,) and higher chromosome number recorded for the monocots is 2*n* = c. 600 in *Voanioala gerardii* (Johnson *et al.*, 1989).

Data about chromosome number of plants help clarify the possible relationship at several levels in the taxonomic works. Since chromosome numbers are generally stable within an organism, observable changes become part of the evolutionary processes leading to speciation (Heslop-Harrison and Schwarzacher, 2011). Once the chromosome numbers of sufficient species are known within a taxonomic group, patterns and trends in the numbers can suggest which specific mechanisms were influential in the development of the group from its ancestral stock (Uhl, 1992). However, there is some confusion in interpretation of the data due to higher diversity in numerical change and recurrence of some karyological mechanisms inside a genus or a family. Species displaying the same chromosome number, ploidy level or structural change are not necessarily closer to each other than species with different chromosome numbers (Guerra, 2008).

A number of plant species are known to have supernumerary chromosomes that do not combine with A chromosomes and follow its own evolutionary pathways (Jones and Houben, 2003). The occurrence of B chromosomes is highly heterogeneous and correlated with genome size (Jones *et al.,* 2008). B's were found in Commelinales (27.2%) and Zingiberales (4.3%) without any chromosome report for non-monocot basal angiosperms (Jones *et al.,* 2008). The two families

with the largest number of B species are Poaceae and Asteraceae. Higher occurrence also existed in Liliales and Commelinales without frequency difference between diploids and polyploids (Levin *et al.,* 2005).

2.8.1.1 Chromosome base number

Chromosome base number can be described as the lowest known haploid number observed in taxon which explains chromosomal variability of a group and its relationship with other closest related groups (Guerra, 2000). Identification of a base number can only be done after detailed analysis of all chromosome numbers are reported for the group (Guera, 2008).

2.8.1.2 Polyploidy

Polyploidy refers to a condition where an organism or a cell possesses more than two sets of basic chromosome numbers (Stebbins, 1971; Chen, 2010). Polyploidy is the most common karyotype variation in plants and consisted of duplication or multiplication of an entire chromosome complement. It is the most common chromosomal mechanism involved in the evolution of plants. Many polyploids are euploids, i.e., they have exact multiples of a monoploid basic number. But, sometimes polyploids have expressed variable degree of dysploidy and often dysploid variants are the only representatives of a ploidy level (Guerra, 2008). An autopolyploid is formed by duplicating a genome within the same species as in potato (*Solanum tuberosum*), alfalfa (*Medicago sativa*), and sugarcane (*Saccharum officinanum*), on the other hand, allopolyploid is the result of hybridization between different species followed by chromosome doubling or from fusion of unreduced gametes between species (Stebbins, 1971; Chen, 2010). Allopolyploids are regarded as major force in evolution while autopolyploids were considered rare (De bodt *et al.,* 2005).

Numerous data from various sources indicated that many of the flowering plant species have faced a polyploid event at some point within the diversification of angiosperms (Cui *et al.,* 2006). On the other hand, these inferences tell us little about the frequency of ongoing polyploidy for specific speciation (Chen, 2010).

Polyploids have the ability to colonize a wider range of habitats with better survival in harsh and unstable climates as compared to their diploid progenitors (Grant, 1981). This may be associated with increased heterozygosity plus genic and biochemical flexibility provided by the presence of additional alleles (Smith *et al.,* 2006). Polyploids contributed less, for development of new adaptive complexes, compared to diploids as a result of 'buffering effect' of multiple genomes, mutations and recombination (De bodt *et al.,* 2005).

2.8.2 Centromere

Centromere is an important landmark for characterization of chromosomes and construction of karyotypes. The majority of plant species have single centromere per chromosome which is termed as monocentric. Some plant species don't possess any localized primary constriction (holocentrics) and their centromeres are considered diffused rather than lacking. This is common in *Carex* and *Luzula* (Stace, 2000). Though rare, holocentric chromosome fragmentation can increase chromosome number, but this is not common in the case for monocentric chromosomes as fragments lacking centromere are lost due to incapability to survive mitosis in a regular way (Stace, 2000).

In conventional cytological analysis, the position of centromere is critical for morphological identification of each chromosome within a cell. Chromosomes can be named as metacentric when the position of centromere is either in the middle or near the middle; sub-metacentric when it is closer to one end; acrocentric in case where the centromere is very close to end and

telocentric if it is located at the end. However, these terminologies have their own limitation as they were used by various authors without clear boundary and even sometimes difficult to understand in a specific case what each term signifies (Levan *et al.,* 1964).

According to Levan *et al.* (1964), centromeric locations are calculated based on the ratio of the long arm (*l*) and the short arm (*s*) expressed as arm ratio index (*r*). Arm ratio index (*r*) is the ratio of long arm length to the short arm length (*r*= l/s). Based on the value obtained through computing arm ratio index, Levan *et al.* (1964) classify chromosomes into six sub-catagories. These include M type (when the centromere is exactly at the center or median point of the chromosome, *r*=1.00), m type (when the centromere is located around the median region, *r*=1.0-1.7), sm (if the centromere is located in sub median region, *r*= 1.7-3.0), st (if the centromere location is in sub-terminal region, *r*=3.0-7.0), *t* (when centromere is located at terminal region, *r*=7.0-∞) and T (if centromere is exactly at terminal point (*censu stricto*), *r*=∞). Most authors use this type of nomenclature either as it is or with slight modification, when describing a particular chromosome or a particular karyotype.

2.8.3 Secondary constriction

Nucleoli are formed and ribosomal RNA is produced in the secondary constriction region of a chromosome. These constrictions are also called nucleolar organising regions (NORs) or r-DNA sites. Mostly, NORs are located sub-terminally and they delimit a short well stained segment of the chromosome distally. The delimited segment is called a satellite with the chromosomes bearing satellites named satellited chromosomes (Stace, 2000).

Chromosomes that bear secondary constriction can be identified by their nucleolus organizer regions and associated satellites. NOR can become clearly visible after staining with silver

nitrate and this is essential for determination of rDNA transcription rate and number and locations of NORs in chromosome complements (Arabbeigi *et al.,* 2011).

2.8.4 Telomere

Telomeres are the physical ends of eukaryotic chromosomes. Several uses are known for telomeres. It is a protective "cap" for chromosome end as broken chromosomes and free DNA ends are prone to end-to-end fusions and exonucleolytic degradation. It also prevents formation of dicentric chromosomes. Furthermore, telomeres help establish the three dimensional architecture of the interphase nucleus and mediate transient associations between homologous and non-homologous chromosomes (Zakian, 1989).

Telomere length is highly variable between species and even significant deviation was there among individuals of a species, tissues of an individual, chromosomes of a single cell and homologous chromosomes (Stindl, 2004). The mean telomere length diversity between species range from less than 1 kbp to several 100 kbp, but seems to be relatively constant within a species (Stindl, 2004).

2.8.5 Chromosome size

The overall combined effect of cellular, molecular, and evolutionary mechanisms can possibly confine species specific average chromosome length (Li *et al.,* 2011). Differences in chromosomal structure and size, in turn, signify the genetic variation in plant species (Arabbeigi *et al.,* 2011). Generally, there is reverse relationship between chromosome number and chromosome size (Verma and Agarwal, 2005).

The variation in absolute length of a particular chromosome of a species or genera can be associated with numerous factors but the main one is the stage of mitosis at the time of fixation

(Dietrich, 1986), because the length of chromosomes depends on their degree of condensation during mitosis.

As to Stebbins (1971), variation in absolute chromosome size of related species or genera possibly reveal the different amount of gene duplication, while segmental interchange involving translocation of unequal chromosomal segments brought the difference in relative chromosome size.

2.8.6 Heterochromatin

Heterochromatin can be constitutive containing satellite DNA and mostly located at the centromere or facultative that becomes inactive in a certain cell lineage but expressed in others (Avramova, 2002). At the beginning of anaphase stage, different chromosomes separate their sister-chromatids at slightly different times. As heterochromatin segment adjacent to centromere becomes larger, the duration for sister-chromatid separation of chromosomes becomes elongated and even at extreme cases this can lead to aneuploidy (Sumner, 2003).

Hereochromatin is stained for various purposes. This can be for identification of heterochromatin sites, study of variation in constitutive heterochromatin as a marker to identify homologues chromosomes and most importantly for identification of individual chromosomes of a particular species (Sumner, 2003).

Generally, identification of particular sites and size of heterochromatin, range of variability and the DNA composition of heterochromatin within a chromosome of a species are the essential aspects of characterizing karyotypes of a species (Sumner, 2003).

2.9. Chromosome staining

The early method of chromosome identification was highly dependent on the shape and size of chromosomes. At that time dyes like carmine, orcein or fuchsin (Feulgen) were used to stain chromosomes which gave strong colour with DNA (Stace, 2000). The difficulty of viewing some chromosome and desire to identify each individual chromosome within a cell led to development of two techniques of chromosome staining, dyes that stain chromosome differently and *insitu* hybridization.

Since the package of chromatid arms along the entire length is not alike, variation in density of each chromosomal region existed. This difference in density is, in turn, utilized by the various banding techniques for visualizing light and dark horizontal (transverse) bands. The diversity of banding patters helps identify chromosomes, locate aberration site and gene mapping (Savage, 2004). Karyotypes of different species may differ in some aspects of their chromosome banding, and so banding can be used to differentiate between species.

The prominently reported patterns like G, C, R and T banding depend on Giemsa staining property of specific chromosomal structure (Drets, 2004). A good quality staining agent has the ability to stain specific chromosomes regions like euchromatin and heterochromatin with clear cytoplasm and nucleoli (Sumner, 2003). Comparing plants with animals, a lot of chromosome banding without modification are well suited for animal chromosomes and not for plant chromosomes (Chaudhary, 2002). *In situ*-Hybridization Techniques use a probe which enables to identify specific regions within a chromosome. It has been reported that that telomere probes can be used to identify centric fusions, chromosome homology and genome evolution (Stace, 2000).

2.9.1 Silver staining

Silver staining is a method of staining nucleolus organizing regions (NORs) with silver nitrate and used for localizing nucleolar organizing region in plant and animal chromosomes (Hizume *et al.,* 1980). It is a technique with extreme effectiveness in detecting intercellular, inter-chromosomal and inter-individual variations in structure and function of NORs (Zurita *et al.,* 1998). Furthermore, the method is also utilized in estimating transcription rate of rDNA in plants (Fatkhutdinova *et al.,* 2002). At the end of mitosis, pre-nuclear bodies is formed and recruited in NORs that finally leads to *de novo* pre-r RNA synthesis and formation of nucleolus in daughter cells (Saez-Vasquez and Medina, 2008).

Silver nitrate stains nucleoli more easily than it does stain the nucleolar organizer region of chromosomes. From the maximum number of nucleoli stained in the interphase or telophase nuclei, it is possible to infer the number of NORs and maximum number of satellies in the genome of a taxon as this is observed in various tissues of *Guizotia abyssinica* (Kifle Dagne and Hennen, 1992).

In polyploid organisms, the number of nucleoli can vary from species to species and even between different tissues depending on percentage of fused and unfused nucleolus during interphase stage (Jackson, 1971). In some instances, the maximum number of nucleoli can indicate ploidy level with additional cytological information like meiosis. But care should be taken not to consider only number of nucleoli per cell as an indication of ploidy level as the nucleolar organizer is a compound structure that subjected to breakage and translocation (Jackson, 1971).

2.9.2 Giemsa staining

Giemsa stain is a mixture of glycerine, methanol, methylene azure and eosin with higher specificity on phosphate groups of DNA and attaches in regions with higher percentage of adening-thymine bonding (Barcia, 2007). The high quality of staining chromatin, ability to identify chromosomal aberrations such as translocation and rearrangement with distinct cytoplasm makes giemsa staining widely applicable in plant cytogenetics (Barcia, 2007). The widely known techniques like C, G, R, T and Q banding used giemsa stain for identification of different chromatic region within achromosome complement (Sumner, 2003).

2.10. Genome size

Genome size is the total amount of DNA contained within a single copy of a genome (Greilhuber *et al.,* 2005). This can be measured in terms of mass (Picograms, Pg) or number of nucleotide base pairs (bp) where 1 pg = 10^9 bp (Whitney *et al.,* 2010). The genome size of organisms is highly diversified as far as about 500-fold variation present among angiosperms (Whitney *et al.,* 2010). In case of diploid species, the smallest genome size (1 c DNA content) was reported in *Arabidopsis thaliana*, n=5 with 0.16 pg (Bennett *et al.,* 2003), while the largest 1 c DNA content was recorded for *Fritillaria davissi* n=12 that contain 90 pg or ~ 157 Mbp (Bennett and Smith, 1976).

Numerous factors can increase or decrease genome size of an organism. Polyploidy, proliferation of introns and amplification of transposable elements, small scale insertion and segmental duplication increase genome size; while deletion and chromosomal loss decrease the genome size (Lynch, 2007). DNA content is often associated with various parameters like cell size, metabolic rate, developmental rate and body size (Gregory, 2004). In plants, for instance,

genome size can affect seed size, pollen development and germination temperature (Lomax *et al.,* 2009).

2.11. Mechanisms of chromosomal polymorphism

Analyzing intra- or inter-species karyotypic variation regarding the structure or number of cytotypes can be essential in determining difference between taxon and inferring patterns of divergence in the population. Such kind of information is also essential for clarifying the possible role of chromosomal rearrangements in evolution and speciation of organisms (Techio *et al.,* 2010).

Due to diversified karyotypes observed in higher organisms, researchers speculate the role of chromosomal change in speciation. Structurally, chromosome rearrangements by inversions, translocations, duplications, deletions, fusions and fissions are well understood and amply explain the origin of karyotype variation resulting from evolutionary divergence (Stace, 2000).

2.11.1 Inversion

Inversions can alter the shape of a particular chromosome. Pericentric inversions involve breakage on either side of centromer with variation in distance, while paracentrics have break point in one arm with difference in distance between a NOR if a chromosome possesses a NORs (Schubert, 2007). Pericentric inversion which involves breakage at either side of a centromere along the length of chromosomes followed by flipping at 180 degree and re-insertion can change the chromosome morphology, for instance, from metacentric/sub-metacentric to telocentric/sub-telocentric or vice versa. The genomic comparison helps to identify inversion polymorphism in species (Hoffman and Reieseberg, 2008).

2.11.2 Deletion, duplication and translocation

Deletion is a loss chromosome segment ranging from micro deletion of few base pairs to an arm or whole chromosome (Coghlan *et al.,* 2005). Deletion can alter chromosome structure if it causes loss of segments on either side of the chromosomal arms and also causes a shift in position of centromere in one direction, for example, from metacentric to sub-metacentric/sub-telocentrics. Change in chromosome number can also be observed if it leads to loss of a whole chromosome (Schubert, 2007).

Duplication is a mechanism of creating additional copy of a segment of chromosome that changes chromosome morphology through increasing size of chromosome arms on either side of a centromere followed by shift in centromere position. Duplication can either be tandem, when the copy is adjacent to the original segment or displaced when located at non-adjacent location. The duplicated segment either has the same orientation, or inverted in which case there is a reverse orientation of the duplicated copy as compared to original (Coghlan *et al.,* 2005).

Translocation is a process of breakage of chromosome segment followed by movement from one location to another within the same chromosome (shift) or to a non-homologous chromosomes in which case the exchange can either be reciprocal or non reciprocal. Equal exchange of chromosome segment between homologous chromosomes is difficult to be noticed as compared to unequal chromosome substitution which can be simply detected by simple karyotyping (Jackson, 1971).

When many breaks exist, segments of chromosomes can be inserted to another pair of chromosome and produce nonreciprocal translocation (Jackson, 1971). This results in net gain of segments to recipient chromosome with loss of the donor. The net addition of segments to

receipient chromosomes leads to an increase in overall size, while the donor's decrease their chromosome size. The exchange in chromosome segments also shifts in position of centromere.

2.11.3 Robertsonian fusion and centric fission

Robertsonian fusion and centric fission are critical in karyotype research as they change chromosome number and morphology. In higher plants, however, such kind of change is rarely found without significant contribution to karyotype variation (Jones, 1998).

Robertsonian fusion is a process of producing a long and short metacentric chromosome after breakage of two non homologous acrocentric chromosomes at the centromere followed by fusion of the long arms to form a large chromosome and the small arms to form a small chromosome which are metacentric or sub-metacentric chromosomes. The initial result will be a change in karyotype without altering the total chromosome number. However, most of the time, the smaller chromosome will subsequently be lost and the total chromosome number will decrease by one from the original. Subsequent Robertsonian fusion leads to steady decrease in chromosome number (Levin, 2002).

Fission involves breakage at the centromere of chromosomes producing two telocentric chromosomes which become stable if telocentric segment is added or results in an increase in chromosome number (Levin, 2002). If centric fission involves an already replicating chromosome it produces isochromosomes. The involvement of fission becomes clearly visible when metacentric of one particular chromosome complement is replaced by two acrocentrics or telocentrics (Jones, 1998).

2.12 Karyotype evolution

The apparently random changes in chromosome set of the cell of particular species, which can be associated with polyploidy, Robertsonian fusion and centric fission, aberration in cell division leading to aneuploidy, can be seen as the first step in speciation through chromosomal evolution (Heslop-Harrison and Schwarzacher, 2011). Polyploidy is probably played a major role in evolution of angiosperms through generating fertile inter-specific hybrids with multiple alleles at each locus, freeing additional genes for mutation and reproductive isolation of new polyploids with limited gene flow (Heslop-Harrison and Schwarzacher, 2011).

The structural change of chromosomes like size, number, banding pattern and shape can occur through chromosomal mutation. The occasional chromosome mutation that might initially exist as polymorphic variant in particular population eventually get lost or fixed through natural selection or random genetic drift. The adaptive mutant variant in a population will establish a reproductive barrier and lead to emergence of new species (Heslop-Harrison and Schwarzacher, 2011).

3. Objectives of the Study

3.1 General objectives

- To study karyotypes of some species of the genus *Commelina* and *Tradescantia* using conventional techniques.

3.2 Specific objectives

The specific objectives of this study are:

- To determine the chromosome number of the species used in the study;
- To determine the ploidy level of these species;
- Construct the karyotype;
- Determine the number of NORs using silver staining;

4. Materials and Methods

4.1 Plant material

Six species of the family Commelinaceae, four species from the genus *Commelina* and two from *Tradescantia* were analyzed cytologically. Species of the genus *Commelina* studied include *Commelina diffusa* (from Jimma, Ginchi and Entoto), *Commelina africana* (from Addis Ababa and Sebeta), *Commelina subulata* (from Ginchi) and *Commelina benghalensis* (from Addis Ababa). Species of *Tradescantia* studied were *Tradescantia fluminensis* (green and variegated forms) and *Tradescantia zebrina* from Addis Ababa (Figure 1). Pictures of representative specimen are presented in Figure 2.

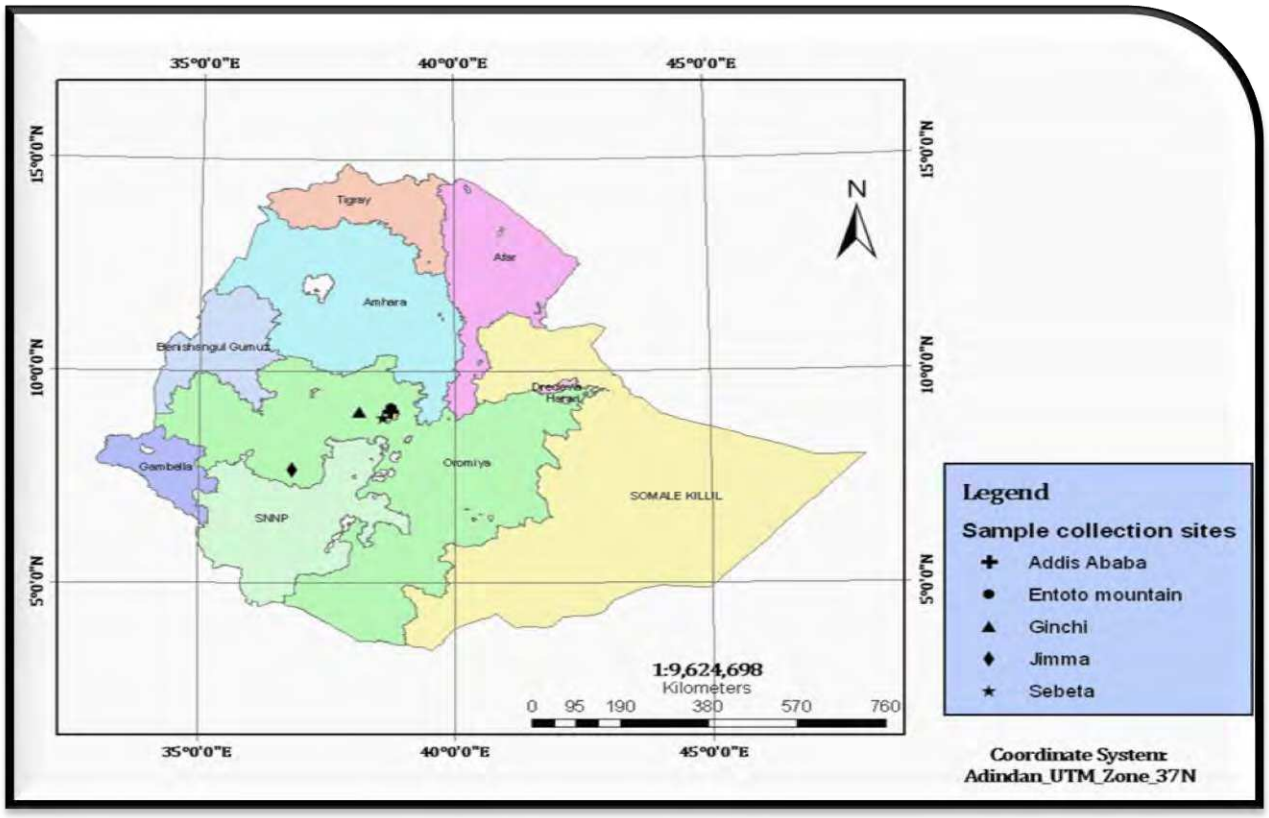

Figure 1. A map of Ethiopia showing the localities from where plant samples were collected for the study.

Table 1. Name of plant species, name of collection sites and collection number of plant specimens used.

Species name	**Collection site**	**Collection number**
Commelina diffusa	Entoto Mountain	ENT 05/11
	Jimma	JIM 10/11
	Ginchi	GIN 06/11
Commelina africana	Addis Ababa (Arat kilo)	AA 05/11
	Sebeta	SEB 06/11
Commelina benghalensis	Addis Ababa (Arat Kilo)	AA 06/11
Commelina subulata	Ginchi	GIN 09/11
Tradescantia fluminensis (green)	Addis Ababa (Arat Kilo)	AA 11/11
Tradescantia fluminensis (variegated)	Addis Ababa	AA 09/11
Tradescantia zebrina	Addis Ababa (Arat Kilo)	AA 07/11

Figure 2. Pictures of plant species used for the study (**A).** *T. zebrina,* (**B**). *T. fluminensis* (green), (**C**). *C. africana* (Addis Ababa), (**D**). *C. benghalensis,* (**E**). *C. diffusa* (Jimma), (**F**). *C. africana* (Sebeta), (**G**). *C. diffusa* (Ginchi), (**H).** *C. diffusa* (Entoto), (**I**). *T. fluminensis* (variegated), and (**J**). *C. subulata*

4.2 Cytogenetic analysis

4.2.1 Somatic chromosome preparation

Chromosome study including numbers and morphology (karyotypes) was done on C-metaphase chromosomes of the root tip meristematic cells. The following techniques were used to prepare and analyse the chromosomes.

4.2.1.1 Pretreatment

Plant specimens collected from different localities were brought to a greenhouse at Faculty of Life Sciences, College of Natural Sciences and planted in pots filled with soil and allowed to grow accordingly. At various times, stems were harvested from the potted plants and allowed to stand submerged in water. In a few days, roots emerged from the nodes of the submerged stems. This provided clean roots for chromosome preparation.

Actively growing roots were harvested from the submerged stems and treated for metaphase arrest of mitosis with the following techniques.

- The harvested roots were immersed in 8-hydroxyquiniline (0.002M) for 3 to 5 hrs
- The roots were then fixed in 3:1 (v/v) of ethanol and glacial acetic acid 1-24 hr at about 4 ^{o}C.
- When they were not needed for immediate slide preparation, the roots were transferred to 70% ethanol and stored at 4^{o}C.

4.2.1.2 Maceration of the roots

- The roots from the fixative or 70% ethanol were rinsed several times in distilled water;
- The roots were then macerated in a solution of pectinase and cellulase in a water bath at 37^{o}C for about 1 hr or more until they were satisfactorily macerated;

- Maceration was stopped when the tips of the root started detaching from the root with or without agitation of the vial;
- The enzyme solution was carefully pipetted out without sucking of the detached root tips and
- Distilled water was added to rinse the root tips, and then transferred to a watch glass or a petridish.

4.2.1.3 Air dry slide preparation

- One or more of the macerated root tips (depending upon the size) were pipetted on a clean slide;
- The water was removed by gently touching the edge of the water drop with a piece of absorbent paper like filter paper or soft paper;
- One or more drops of fresh fixative (3:1, ethanol: acetic acid) were added to the root tips, and the tips were mashed quickly with flat ended mounted needle; and
- The slide was then allowed to air dry at room temperature and stored until needed for staining.

4.2.1.4 Slide staining

- Slides with good preparations were screened under phase contrast microscope before staining;
- The air-dry slides with promising preparation were stained in Giemsa stain in Sorenson phosphate buffer solution (PH 6.8);
- When correct contrast of staining was obtained, the slides were rinsed in distilled water, and allowed to air-dry at room temperature at least for 24 hrs; and

- The preparation was made permanent by mounting in depex mountant and the gum was allowed to set for several days before examining under the microscope.

4.2.1.5 Photography and chromosome analysis

- The slides were analyzed under the microscope for cells containing well spread chromosomes;
- Pictures of the chromosome were taken using a camera fitted microscope; and
- Enlarged photomicrograph prints were made and used for chromosome analysis including karyotype construction.

4.2.2 Silver staining

For silver staining of nucleoli the following procedure was followed

- The slide was prepared similarly with the above procedure for somatic chromosome study except, in this case, the freshly collected root tips were directly fixed in 3:1 (ethanol to acetic acid) without treatment with 8-hydroxyquiniline. The latter treatment was omitted in order to obtain telophase cells during which the maximum number of nucleoli may be observed.
- An aqueous silver nitrate solution was then prepared by mixing 1 gram of Ag NO_3 in 1 ml double distilled water plus 10 µl formalin following Tezera Temesgen, (2007);
- Drops of silver nitrate solution were added to the air dried slide preparation and this was covered with 25x40 mm clean piece of nylon cloth;
- The nylon covered slide was placed in a petridish lined with wet filter paper (filter paper wetted with distilled water);

- The petridish was incubated in an oven at 60^{O}C for about 2 hours with frequent follow-ups and adding of more drops of silver nitrate solution on the slide, and more water on the filter paper to maintain wet condition in the petridish;
- The resulting slide was rinsed in double distilled water followed by air drying; and
- Slides were examined under a microscope and pictures of good nucleoli were taken for subsequent analysis.

4.2.3 Karyotype analysis

The printed pictures of chromosomes were scanned into computer and the lengths of the whole chromosomes and their arms were measured in terms of pixel per cm using micro measure computer software version 3.3. Accordingly, the arm ratio of the chromosomes was calculated by dividing the length of the long arm to that of short arm.

Karyotypes were constructed by cutting and arranging the putative homologous chromosomes into pairs based on arm ratio(*r*) and chromosome size using the Smart Type software version 0.8. Chromosomes were categorized into chromosome types based on arm ratio (*r*) according to Leval *et al.* (1964) with slight modification. In the present case the term metacentric chromosome was used to include both M and m types with *r* =1.0-1.7. Sub-metacentric was used as synonymous with sub-median chromosome of *r*= 1.7-3.0 and st is similar to sub-telocentric when *r*=3.0-7.0.

After accurate measurement of karyotyped chromosomes were obtained, intra-chromosomal asymmetry (A_1) and inter-chromosomal asymmetry (A_2) indices have been calculated using Romero Zarco (1986) method:

$$A_1 = 1 - \frac{\sum_{i=1}^{n} bi/Bi}{n}$$

Where b_i=average length of short arms in every homologous chromosome pair, B_i= average length of long arms of every homologous pairs and n= number of homologous chromosomes

$$A_2 = \frac{Sd}{x}$$

Where Sd = Standard deviation of chromosome length and x =mean chromosome length

DI (dispersion index) of chromosomes was also calculated according to the method devised by Lavania and Srivastava (1999) that depends on centromeric gradient (C_G) and coefficient of variation in chromosome length (C_V)

$$C_G = \frac{Msa}{Mla} \times 100$$

Where Msa = median length of short arms, and Mla = median length of long arms

$$C_V = A_2 \times 100$$

$$DI = \frac{CG \times CV}{100}$$

Measurements like intra chromosomal (A_1), inter-chromosomal (A_2), dispersion indices (DI) and Stebbins asymmetry depends on both chromosome size and centromeric position for estimating karyotype asymmetry of chromosomes. But asymmetry index depends only on centromeric position (Kumari and Roy, 2010).

5. Results

Chromosome count, basic chromosome number, ploidy level, number of metaphase plates counted and karyotypic formula are presented in Table 2. In addition, data on chromosome length, average chromosome length and Stebbins asymmetry are presented in Table 3. Inter-chromosomal asymmetry index, intra-chromosomal asymmetry index, coefficient of variation in chromosome length, centromeric gradient and dispersion index are presented in Table 4.

Karyotypic analysis based on chromosome count, length of each chromosome within a plant and arm ratios were presented in Appendices 1-9. As presented in Table 2, the *Commelina* species contain metacentric, sub-metacentric and sub-telocentric chromosomes with variation in number and morphology, while *Tradescantia* possess telocentric chromosomes in addition to the above listed chromosome types.

Table 2. Species name, somatic chromosome number (2*n*), basic number (*x*), ploidy level and karyotype formula of the study plants

Species Name	**2*n***	**Basic number (*x*)**	**Ploidy level**	**Karyotype Formula**	**Number of metaphase plates counted**
C. africana (Addis Ababa)	30	15	2*x*	12m + 10sm + 8st	10
C. africana (Sebeta)	30	15	2*x*	14m + 10sm + 6st	4
C. benghalensis	66	11	6*x*	36m + 24sm + 6st	4
C. diffusa (Entoto)	30	15	2*x*	16m + 6sm + 8st	8
C. diffusa (Ginchi)	66	-	-	28m + 26sm + 12st	5
C. diffusa (Jimma)	30	15	2*x*	10m + 8sm + 12st	10
C. subulata	30	15	2*x*	18m + 10sm + 2st	4
T. zebrina	24	12	2*x*	4m + 6st + 14t	4
T. fluminensis (variegated)	60	-	-	-	3
T. fluminensis (green)	60	-	-	8m + 6sm + 22st + 24t	6

Table 3. Range in chromosome length, average chromosome length (**ACL**), **K,** percentage of chromosome with ratio=r>2; **L,** ratio of largest to smallest chromosome; **M,** degree of asymmetry and Stebbins asymmetry index of the studied species of Commelinaceae.

Species	**Chromosome length (µm)** **Shortest: longest**	**ACL (µm)**		**Stebbins asymmetry**		
			Asymmetry index	**K**	**L**	**M**
C. africana (A.A)	4.53 – 7.5	5.89	65.19	0.43	1.67	2A
C. africana (Sebeta)	2.66 – 7.34	4.64	65.89	0.33	2.76	2B
C. benghalensis	2.5 – 5.625	4.06	62.58	0.35	2.25	2B
C. diffusa (Entoto)	4.167 – 8.125	5.99	66.52	0.467	1.95	2A
C. diffusa (Ginchi)	4.375 – 11.25	7.69	65.56	0.5	2.57	2B
C. diffusa (Jimma)	2.5 – 4.58	3.47	69.497	0.67	1.832	3A
C. subulata	1.923 – 3.654	2.772	60.232	0.267	1.9	2A
T. zebrina	7.031- 19.0625	11.0397	77.375	0.83	2.71	3B
T. fluminensis (green)	1.83 – 6.154	3.096	81.02	0.67	3.345	3B

Table 4. Intra-chromosomal asymmetry index **A1**, inter-chromosomal asymmetry index **A2**, Coefficient of variation of chromosome length $\mathbf{C_v}$, ratio of centromeric gradient $\mathbf{C_G}$ and dispersion index **DI** of the studied species of family Commelinaceae

Species	$\mathbf{A_1}$	$\mathbf{A_2}$	$\mathbf{C_v}$	$\mathbf{C_G}$	**DI**
C. africana (Addis Ababa)	0.4345	0.1498	14.98	55.32	8.29
C. africana (Sebeta)	0.4415	0.21468	21.468	55.102	11.83
C. benghalensis	0.3715	0.1582	15.82	57.831	9.15
C. diffusa (Ginchi)	0.435	0.17035	17.035	53.0163	9.031
C. diffusa (Entoto)	0.4629	0.19223	19.223	51.6155	9.92
C. diffusa (Jimma)	0.76717	0.13771	13.771	39.32	5.415
C. subulata	0.3523	0.1891	18.91	67.45	12.755
T. zebrina	0.73174	0.2931	29.31	11.983	3.5122
T. fluminensis (green)	0.7252	0.3278	32.78	22.93	7.52

5.1 *Commelina africana*

5.1.1 *C. africana* from Addis Ababa

Somatic chromosomes observed from more than five intact cells showed that *C. africana* collected from Addis Ababa contained a diploid number of $2n=2x= 30$. Because of its better chromosome spread and clear chromosome morphology a cell with 29 chromosome number is shown in Figure 3A and a karyotype constructed from same cell in Figure 3B. This variant of the species consisted of six pairs of metacentric chromosomes (pairs 1 to 6), five pairs of sub-metacentic chromosomes (pair numbers 7 to 11) and four sub-telocentrics (pairs 12 to 15) (Figure 3B). The karyotype formula is, therefore, 12m + 10sm + 8 st (Table 2). According to Stebbins karyotype classification, this species belongs to 2A type with asymmetry index of 65.19 (Table 3). Small satellites were also observed at the tip of the short arm of a pair of chromosomes (Figure 3C) and these correspond to the 8^{th} pair on the karyotype (Figure 3B). Figure 3D shows three interphase nucleoli, from which it can be assumed that the maximum number of nucleoli and so satellites for this species is four.

The length wise measurement of metaphase chromosomes revealed a value range from the smallest 4.53µm to largest 7.5µm (Table 3 and Appendix1). Total length of whole chromosome complement ($2n$) was 176.72µm (Table 3 and Appendix1). The entire length of long arm chromosomes for diploid set was 115.2µm and the total length of short arms for diploid set was 61.52µm (Appendix 1). The chromosomes were medium in size with an average length and asymmetry of 5.89µm and 1.87, respectively.

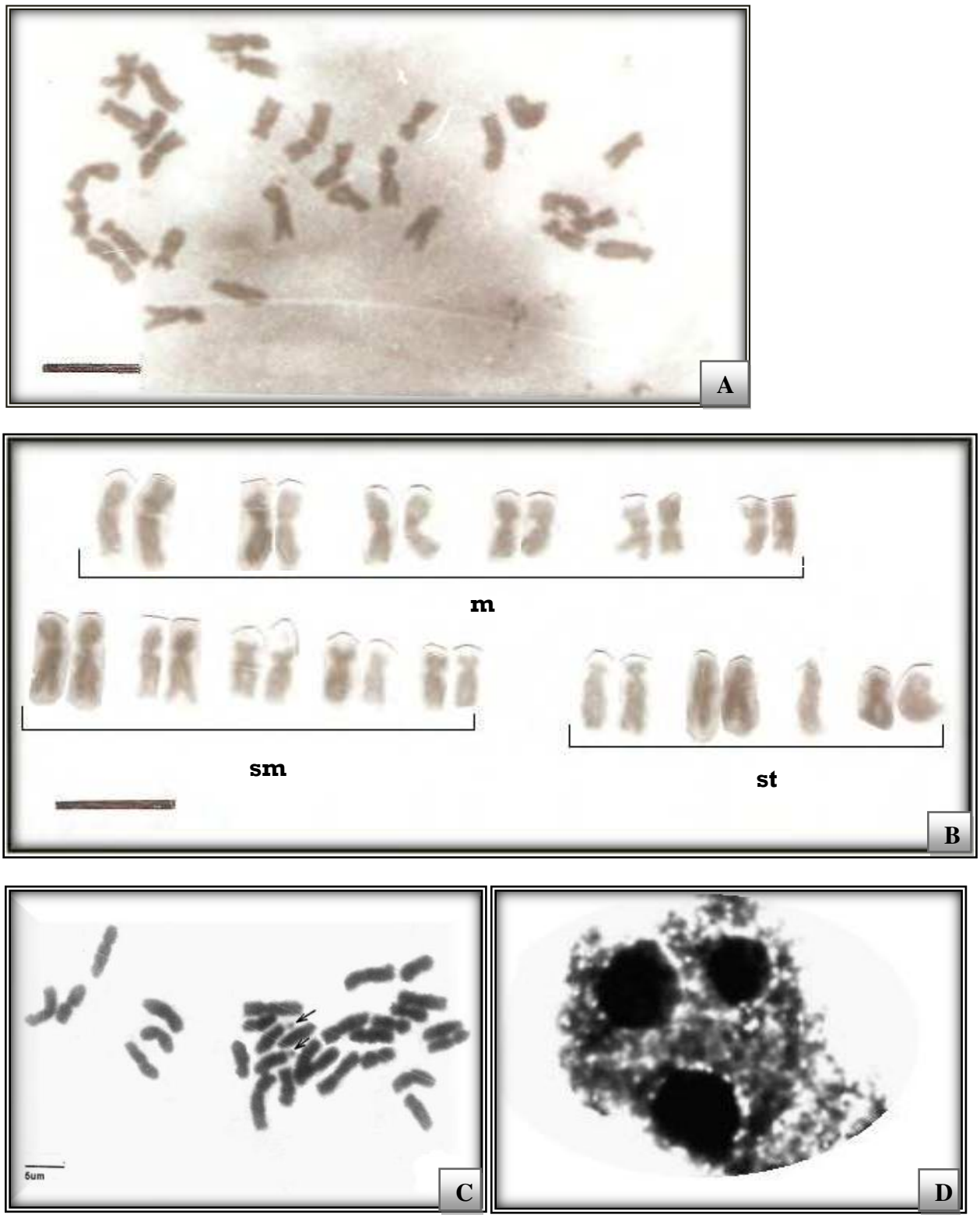

Figure 3. Somatic metaphase spread, karyotype and nucleoli of *C. africana* (2*n*=30) collected from Addis Ababa (A) metaphase spread, (B) karyotype, (C). somatic spread sharing satellites (arrows) and (D). telophase nucleus with three nucleoli. Scale bar = 10µm

This particular specimen of *C. africana* has an intra-chromosomal asymmetry index and inter-chromosomal asymmetry index of 0.4345 and 0.1498, respectively. As presented in Table 4, *C. africana* (Addis Ababa) possesses a ratio in centromeric gradient (55.32), coefficient of variation in chromosome length (14.98) and dispersion index (8.29) (Table 4).

5.1.2 *C. africana* from Sebeta

As to the result obtained from metaphase spread, this specimen possesses $2n=2x=30$ chromosomes (Figure 4 and Table 2). Karyotype constructed based on chromosome length and arm ratio (Appendix 4) revealed the presence of fourteen metacentrics (pair numbers 3, 4, 7, 8, 9, 11, 14), ten sub-metacentrics (pair numbers 1, 6, 10, 13, 15) and six (pair number 2, 5, 12) sub-telocentrics (Figure 4B). Hence the karyotype formula was $2n= 14m + 10sm +6st$. According to Stebbins classification, *C. africana* (Sebeta) has a karyotype index of 65.89 and it is 2B in asymmetry type (Table 3). Four satellited chromosomes and a maximum of four interphase nucleoli were obtained in Figure 4(C and D).

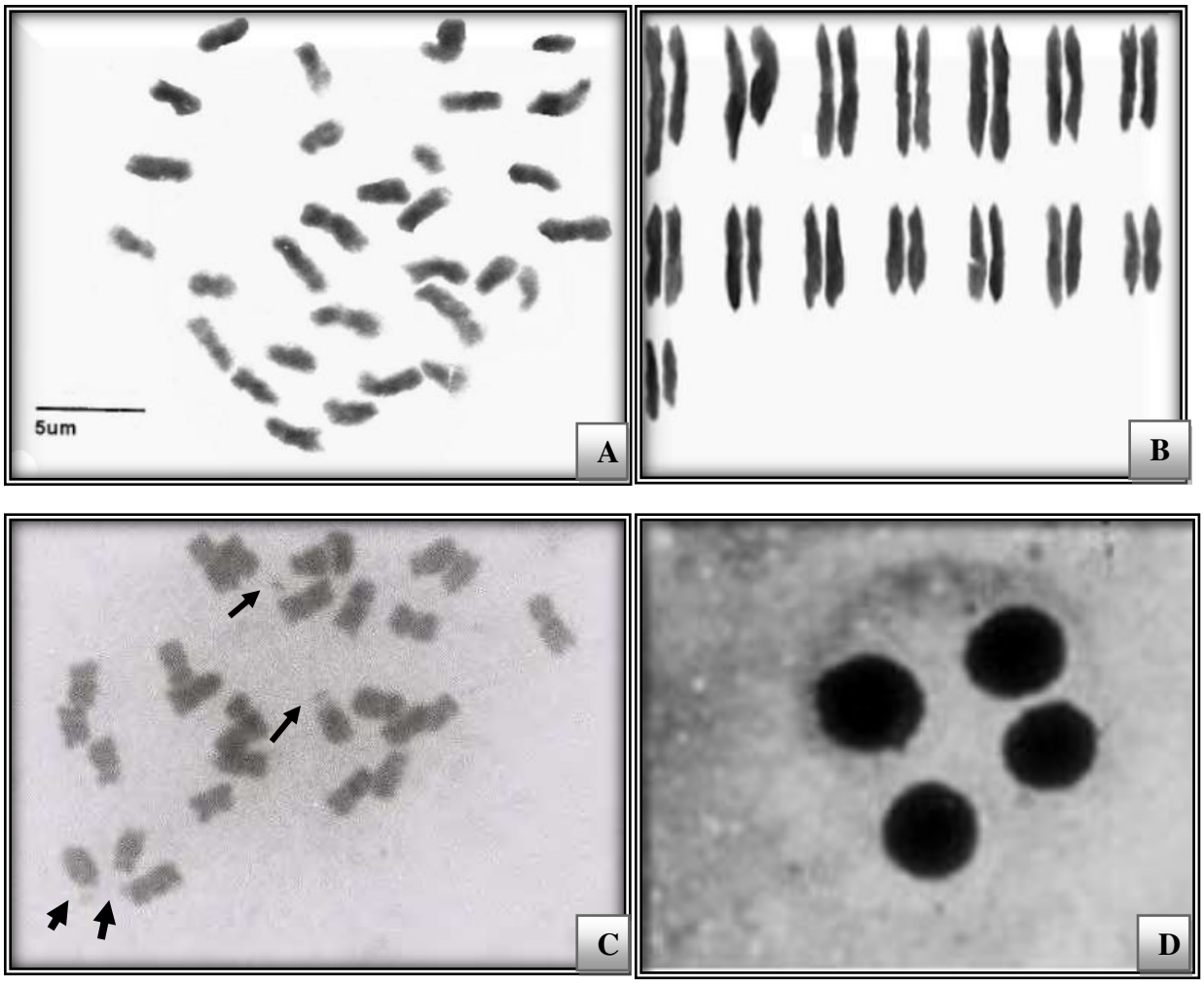

Figure 4. Somatic metaphase chromosomes spread, karyotype and nucleoli of *Commelina africana* collected from Sebeta. (A). metaphase spread, (B). karyotype, (C). four satellites with variation in arrow type indicating homologoues satellited chromosomes and (D). four interphase nucleoli. Scale bar = 5µm

The chromosome length varies from smallest 2.66µm to the largest 7.34µm (Table 3 and Appendix 5). The total length of whole chromosome complement was about 139.18µm (Table 3 and Appendix 5).The total length of long arms and short arms for the diploid set was 91.71µm and 47.47µm, respectively (Appendix 4). The chromosomes were short to medium in size with an average length of 4.64µm. Generally, this species has an average asymmetry of 1.93.

Based on Romero Zarco (1986) formula of calculating intra-chromosomal asymmetry and inter-chromosomal asymmetry indices, this particular species possesses 0.4415 and 0.21468,

respectively (Table 4). *C. africana* (Sebeta) consisted of a coefficient of variation in chromosome length (21.468), ratio of centromeric gradient (55.102) and dispersion index (11.83) (Table 4)

5.2 Commelina benghalensis

According to somatic chromosome analysis of the root tips, this species possesses $2n=6x=66$ (Figure 5 and Table 2). The karyotpe constructed based on calculated arm ratio (Appendix 2) and chromosome size revealed the presence of eighteen metacentric chromosome pairs (pair numbers 1, 3, 4, 5, 8, 11, 14, 15, 16, 18, 22, 24, 25, 29, 30, 31, 32, 33), twelve sub-metacentrics (pair numbers 6, 7, 9, 10, 12, 17, 19, 20, 21, 23, 27, 28) and 3 sub-telocentric pairs (pairs 2, 13, 26) (Figure 5B). The karyotype formula was, therefore, 36m + 24sm + 6st. As to Stebbins method of classification, *C. benghalensis* (Addis Ababa) possesses asymmetry index of 62.58 with 2B in karyotype asymmetry (Table 3). A maximum of five nucleoli were observed through silver staining but no satellites were observed (Figure 5C). Two of the nucleoli are much larger than the other three nucleoli. Each of the larger nucleoli might have resulted from fusion of two or more nucleoli. It can be inferred that six or more satellited chromosomes are present in this species.

The chromosome lengths differ from the smallest 2.5µm and gradually increased upto the largest 5.625µm (Table 3 and Appendix 2). The total length of whole chromosome complement was about 267.935µm (Table 3 and Appendix 2). The total length of the long arms of the diploid set was 167.675µm and that of short arms for diploid set was 100.26µm with their ratio generating an average karyotype asymmetry of 1.672 (Appendix 2). Generally, *C. benghalensis* (Addis Ababa) has predominantly metacentric and sub-metacentric type of chromosomes. The chromosomes were short to medium in size with an average length of 4.06µm.

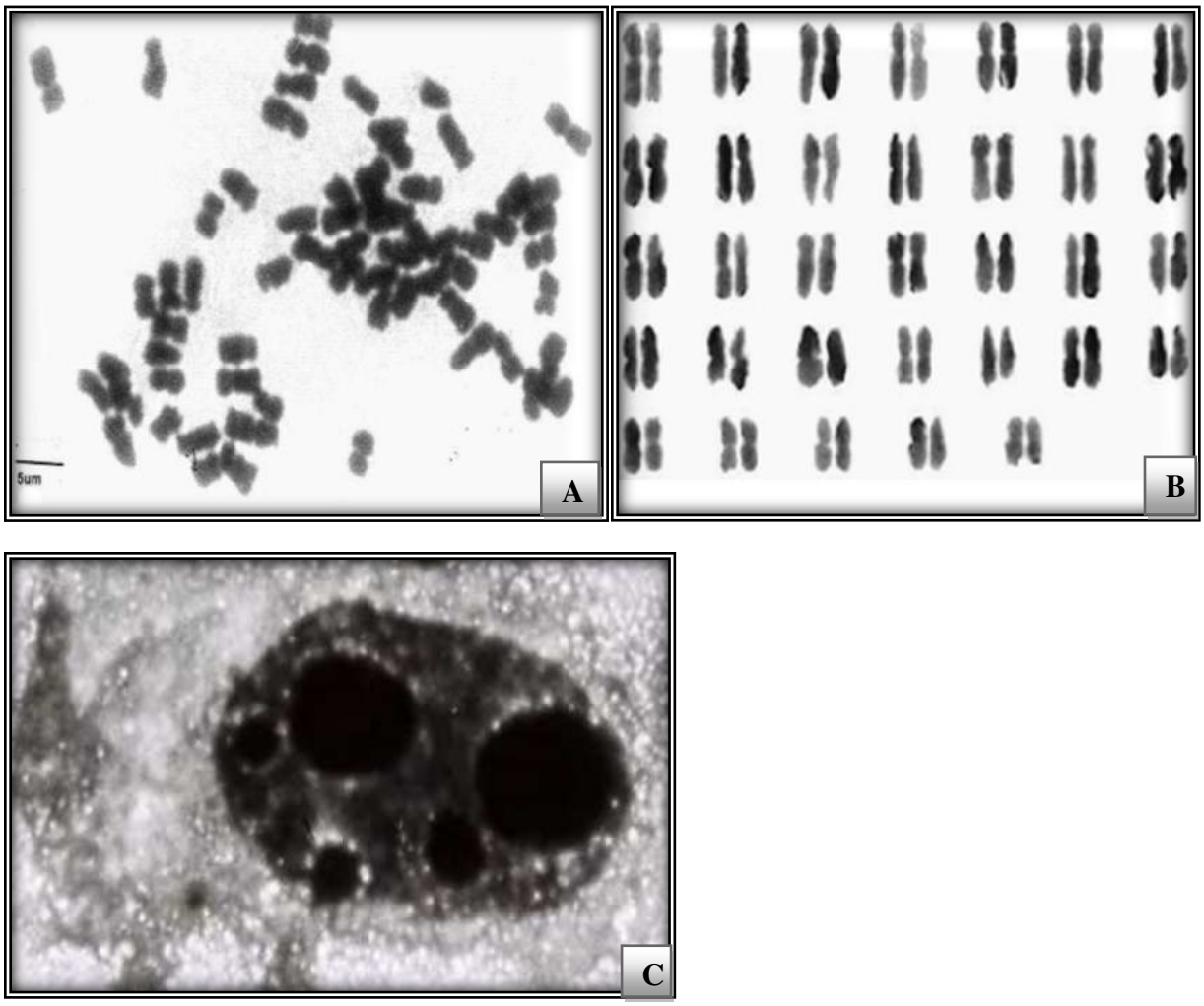

Figure 5. Metaphase chromosomes spread, karyotype and nucleoli of *Commelina benghalensis* (A). metaphase chromosomes, (B). karyotype and (C). five interphase nucleoli. Scale bar = 5µm *Commelina benghalensis* has intra-chromosomal asymmetry index (0.3715) and inter-chromosomal asymmetry index (0.1582) (Table 4). The species also possesses a ratio in centromeric gradient, coefficient of variation of chromosomes length and dispersion index of 57.831, 15.82 and 9.15 respectively (Table 4).

5.3 Karyotype description of *Commelina diffusa*

5.3.1 *C. diffusa* from Entoto

This variant consisted of 2*n*= 2*x*=30 chromosomes (Table 2 and Figure 6). Among a total of 15 pairs of chromosomes, the karyotpe constructed using centromeric ratio and chromosome size (Appendix 5) revealed the presence of sixteen metacentrics (pair numbers 1 to 8), six sub-metacentrics (pair numbers 9 to 11) and eight sub-telocentric chromosomes (pair numbers 12 to 15). The karyotpe formula was 16m + 6sm + 8st. The asymmetry index of this species is also 66.52 with 2A in type (Table 3). A total of four satellites and a maximum of four telophase nucleoli were observed in Figure 6(C and D). One pair has relatively small satellites and the other pair is relatively larger (Figure 6C).

Chromosome lengths vary from shortest 4.167µm to longest 8.125µm (Table 3 and Appendix 5). The total length of whole chromosome complement was 179.715µm with summation of long arms for the diploid set is 119.55µm and total length of short arms for diploid set is 60.165µm (Table 3 and Appendix 5). This species has higher average karyotype asymmetry as compared to the other studied *Commelina* plants with a value of 2.01 (Appendix 5). Generally the chromosomes are medium in size with an average length of 5.99µm.

As to calculation of intra-chromosomal asymmetry and inter-chromosomal asymmetry indices, this particular species has a value of 0.4629 and 0.19223 respectively (Table 4). *C. diffusa* (Entoto) have a coefficient of variation in chromosome length (19.22), ratio of centromeric gradient (51.6155) and dispersion index (9.92) (Table 4).

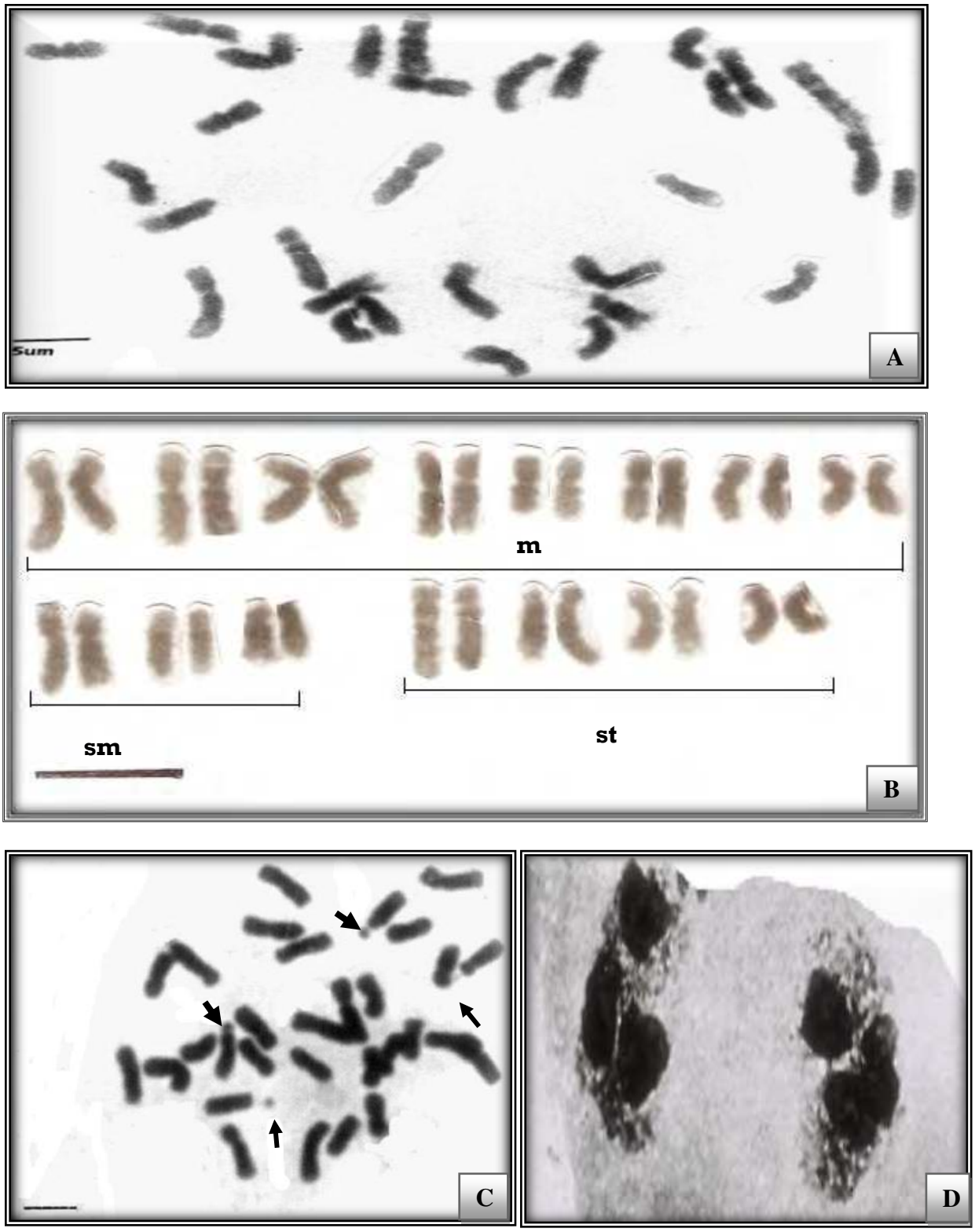

Figure 6. Metaphase chromosomes spread, karyotype and nucleoli of *Commelina diffusa* of Entoto mountain (A). metaphase chromosomes, (B). karyotype, (C). satellites, (small araws, and large arms head) and (D). two telophase nuclei with three and four nucleoli. Scale bar = 10µm

5.3.2 *C. diffusa* from Ginchi

The variant of *C. diffusa* collected from Ginchi possessed 2*n*= 66 (Table 2 and Figure 7). Based on centromeric ratio and chromosome length measurements (Appendix 3), the karyotype (Figure 7B) consisted of fourteen metacentric pairs (pair numbers 1, 4, 6, 15, 16, 20, 21, 23, 25, 26, 30, 31, 32, 33), thirteen sub-metacentrics (pairs 2, 3, 5, 7, 8, 9, 10, 13, 17, 19, 24, 27, 28) and six sub-telocentrics (pairs 11, 12, 14, 18, 22, 29) with a formula of 28m + 26sm + 12st. The asymmetry index is 65.56 and belongs to 2B type of Stebbins karyotype classification (Table 3). As shown in Figure 7(C and D), a total of six satellites and four nucleoli were observed from chromosome preparation and silver staining, respectively. Size of satellites varies with one pair being larger than the other two.

Commelina diffusa (Ginchi) has comparatively larger chromosomes than the other *Commelina* species included in this study with a length range from smallest 4.375µm to largest 11.25µm and total length of whole chromosome complement 507.53µm (Table 3 and Appendix 3). The total length of long arms and short arms for the diploid set was 332.745µm and 174.785µm respectively (Table 3 and Appendix 3). The average karyotype asymmetry is 1.904 (Appendix 3) and on the average the chromosomes can be classified under sub-metacentric. The chromosomes were medium to large in size with an average length of 7.69µm.

C. diffusa (Ginchi) has intra-chromosomal and inter-chromosomal asymmetry indices of 0.435 and 0.17035 respectively (Table 4). This variant also has a coefficient of variation in chromosome length of (17.035), ratio of centromeric gradient (53.0163) and dispersion index (9.031) (Table 4).

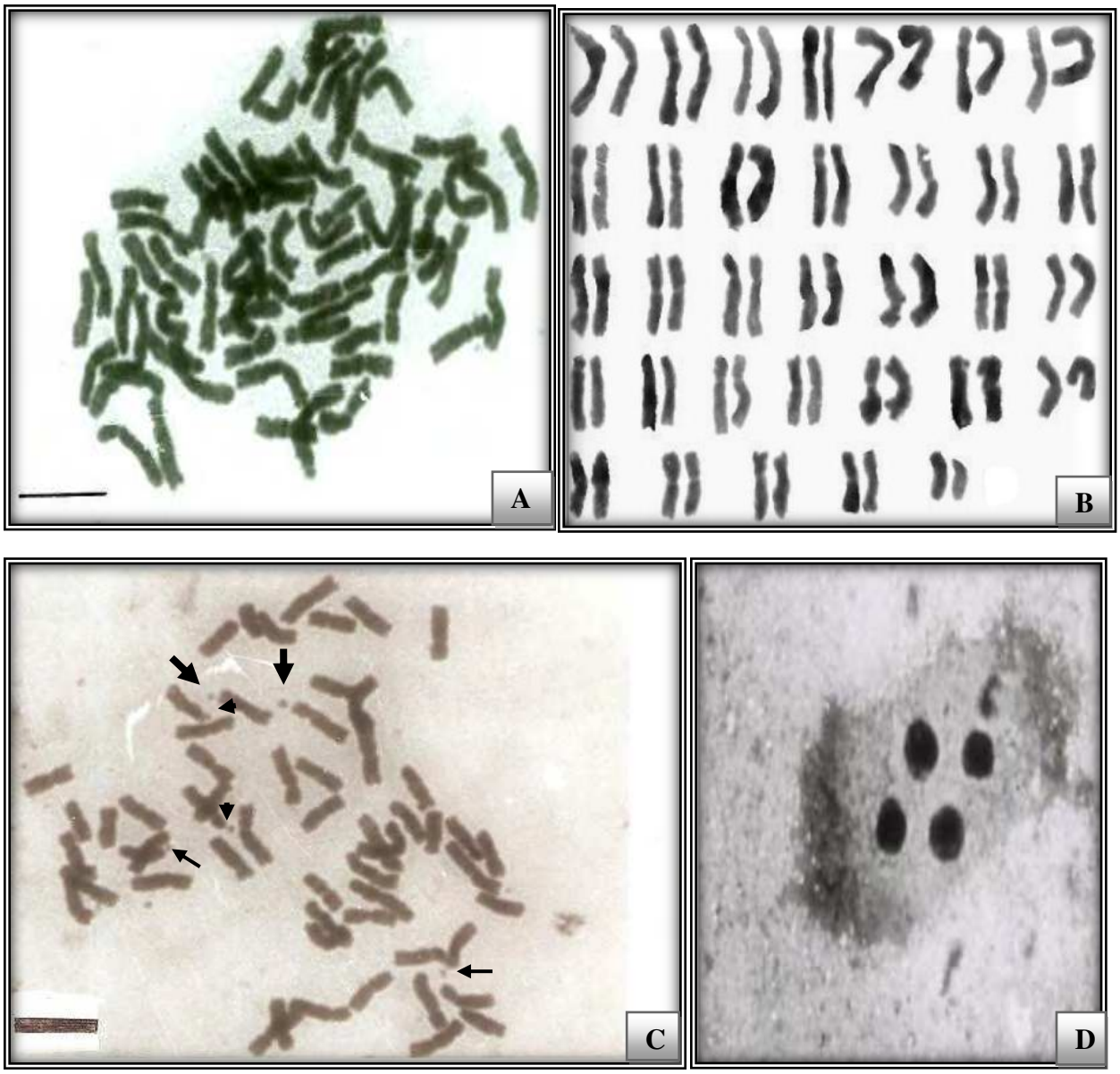

Figure 7. Metaphase chromosomes, karyotype and nucleoli of *Commelina diffusa* from Ginchi. (A). metaphase chromosome spread, (B). karyotype, (C). partial metaphase chromosome spread to show satellites and (D). Nucleoli. Scale bar = 10µm

5.3.3 *C. diffusa* from Jimma

The material of this species collected from Jimma possessed $2n=2x=30$ (Table 2 and Figure 8). According to centromeric ratio and chromosome length measurements (Appendix 6), the karyotype (Figure 8B) has five metacentric pairs (pair numbers 1 to 5), 4 sub-metacentric pairs

(pairs 6 to 9) and 6 sub-telocentric pairs (pair numbers 10 to 15) with a formula of 10m + 8sm + 12st. The asymmetry index is comparatively higher than the other studied *Commelina* species with a value of 69.497 and according to Stebbins karyotype asymmetry classification, *C. diffusa* (Jimma) belongs to 3A type (Table 3). Even if no satellites were observed from the current plant material, a total of three interphase nucleoli were observed that possibly predict the number of NORs or satellites to be four (Figure 8C).

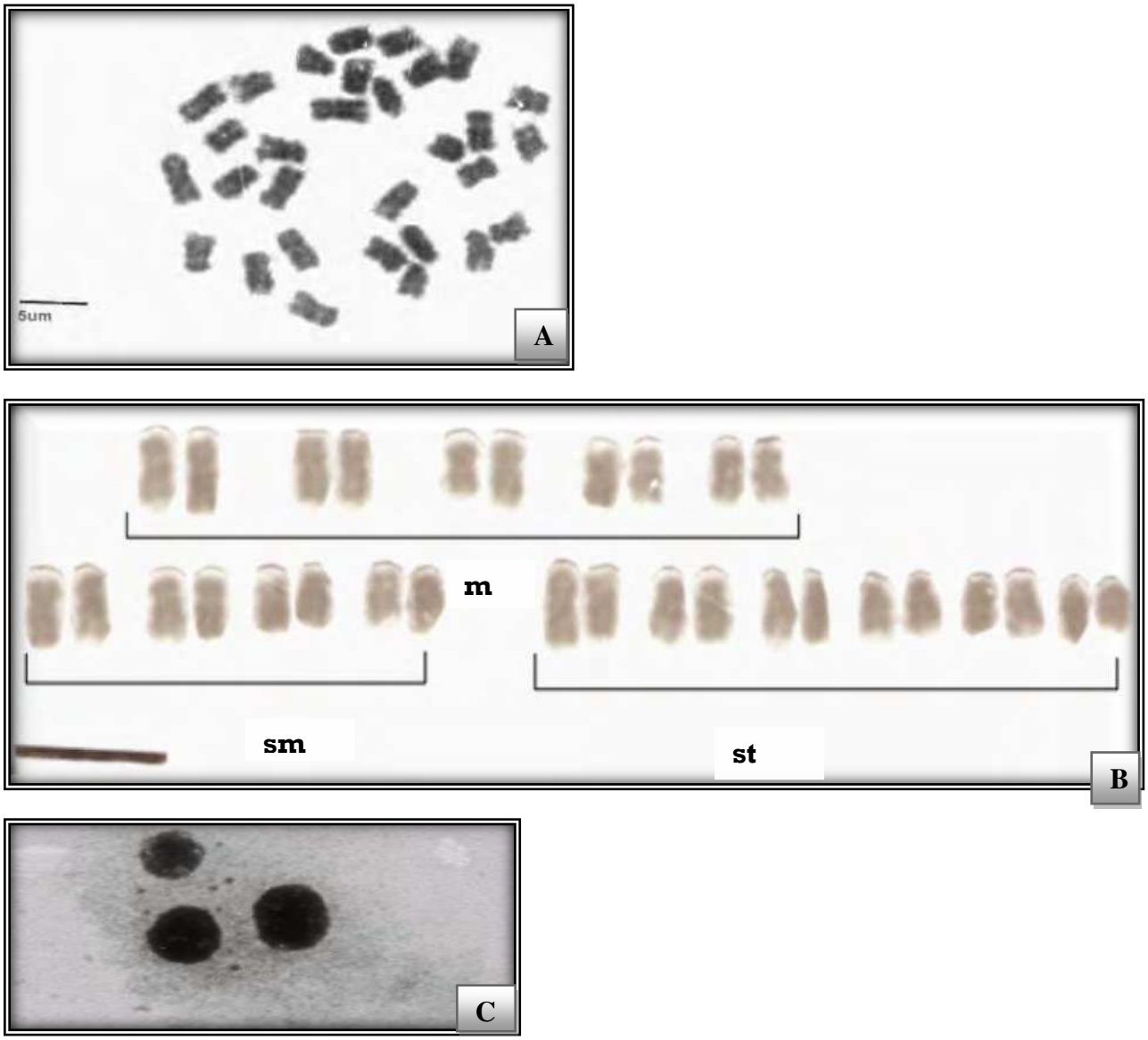

Figure 8. Metaphase chromosomes, karyotype and nucleoli of *Commelina diffusa* from Jimma (A). metaphase spread, (B). karyotype and (C). three nucleoli. Scale bar = 10µm

Commelina diffusa (Jimma) possesses the smallest chromosomes size next to *C. subulata* with a length range from shortest 2.5µm to longest 4.58µm and total length of whole 2*n* chromosome complement 104.22µm (Table 3 and Appendix 6). The total length of long arms for the diploid set was 72.43µm and total length of short arms for the diploid set was 31.79 (Appendix 6). The chromosomes range from very small to medium in size with an average length of 3.47µm and karyotype asymmetry of 2.28µm.

This particular variant has the highest value in terms of intra-chromosomal asymmetry index (0.76717). It also possesses the lowest inter-chromosomal asymmetry index (0.13771), coefficient of variation in chromosome length (13.771), ratio in centromeric gradient (39.32) and dispersion index (5.415) when compared to values from all other studied plants of the genus *Commelina*.

5.4 *Commelina subulata*

The experimental species collected from Ginchi indicated that *C. subulata* has a diploid number of 2*n* =2*x*= 30 (Table 2 and Figure 10). As to arm ratio and chromosome length (Appendix 8) measurements, the karyotype (Figure 10B) consisted of nine metacentric pairs (pair numbers 1, 2, 3, 5, 8, 9, 10, 12, 13), five sub-metacentrics (4, 6, 11, 14, 15) and one pair of sub-telocentrics (7) with a karyotypic formula of 18m + 10sm + 2st. The species possesses the lowest value in asymmetry index (60.232), being the 2A type in Stebbins asymmetry classification (Table 3). Two interphase nucleoli were observed (Figure 10C), but no satellies were observed. One of the two nucleoli (Figure 10C) is larger than the other, which could result from fusion of two or more nucleoli; and it may be inferred that a total of four satellite chromosomes/ NORs are present.

Commelina subulata (Ginchi) has the smallest chromosome size than other species included in this study with a length range from smallest1.923µm to largest 3.654µm and the total length of

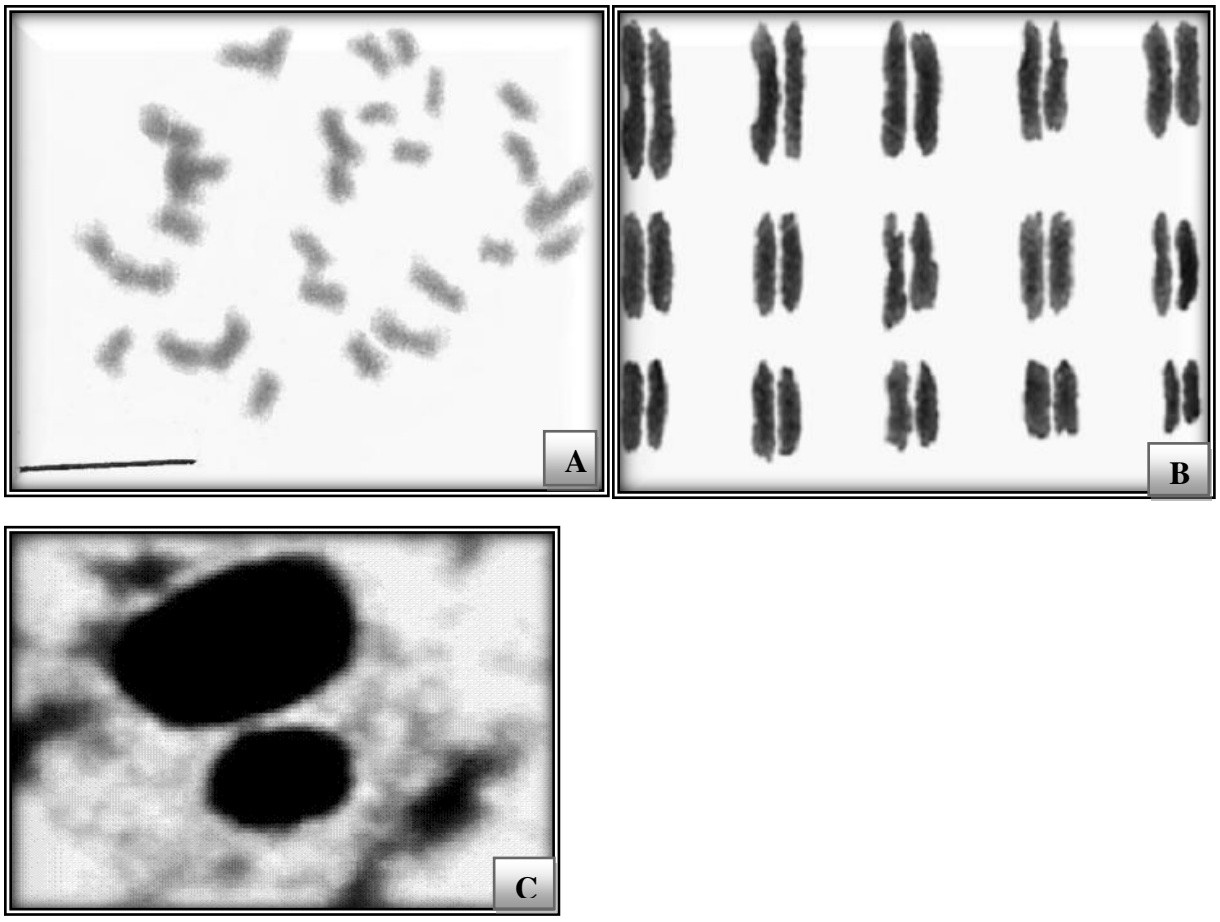

Figure 9. Metaphase chromosomes spread, karyotype and nucleoli of *C. subulata* collected from Ginchi (A). metaphase spread, (B). karyotype and (C). two nucleus having two nucleoli. Scale bar = 5µm

whole chromosome complement is 83.154µm (Table 3 and Appendix 8). The total length of long arms and short arms for the diploid set was 50.085µm and 33.069µm, respectively (Table 3 and Appendix 10). The average karyotype asymmetry is 1.51 (Appendix 8) and the chromosomes, on the average arm ratio, can be considered as metacentric. The chromosomes are very small to medium in size with an average length of 2.77µm.

Commelina subulata possesses the lowest intra-chromosomal asymmetry index, highest ratio of centromeric gradient and dispersion index with a value of 0.3523, 12.755 and 67.45, respectively. The species also consisted of an inter-chromosomal asymmetry index (0.1891) and coefficient of variation in chromosome length (18.91) (Table 4).

5.5 *Tradescantia fluminensis* (green)

Chromosome number for the diploid set was 2*n*= 60 (Table 2 and Figure 11). The karyotype prepared based on centromeric position and chromosome length (Appendix 9) indicated the presence of eight median chromossomes (pairs 6, 13, 19, 25), six sub-median chromosomes (pair numbers 14, 20, 24), twenty two sub-terminals (pairs 2, 4, 8, 9, 10, 12, 17, 18, 21, 29, 30) and twenty four terminals (pair numbers 1, 3, 5, 7, 11, 15, 16, 22, 23, 26, 27, 28) centromers with karyotype formula of 8m + 6sm + 22st + 24t. The asymmetry index is of highest number compared to other studied species (81.02) and Stebbins karyotype classification were 3B (Table 3). This species possesses bimodal karyotype.

No satellites were observed during the study period. In Figure 10(A and C), biarmed chromosomes cannot be easily distinguished. Some of these chromosomes are shown (Figure 10C). A total of three nucleoli with variation in size were observed, from which it can inferred that the possible number of satellites is atleast four (Figure 10D).

The chromosome lengths ranged from smallest 1.83µm upto largest 6.154µm (Table 3 and Appendix 9) with total length of whole chromosome complement 185.77µm (Table 3 and Appendix 9). The total lengths of long arms and short arms for the diploid set were 150.51µm and 35.26µm respectively with an average karyotype asymmetry of 4.27. Generally *Tradescantia fluminensis* (green) has predominantly terminal and sub-terminal chromosomes with an average length of 3.096µm.

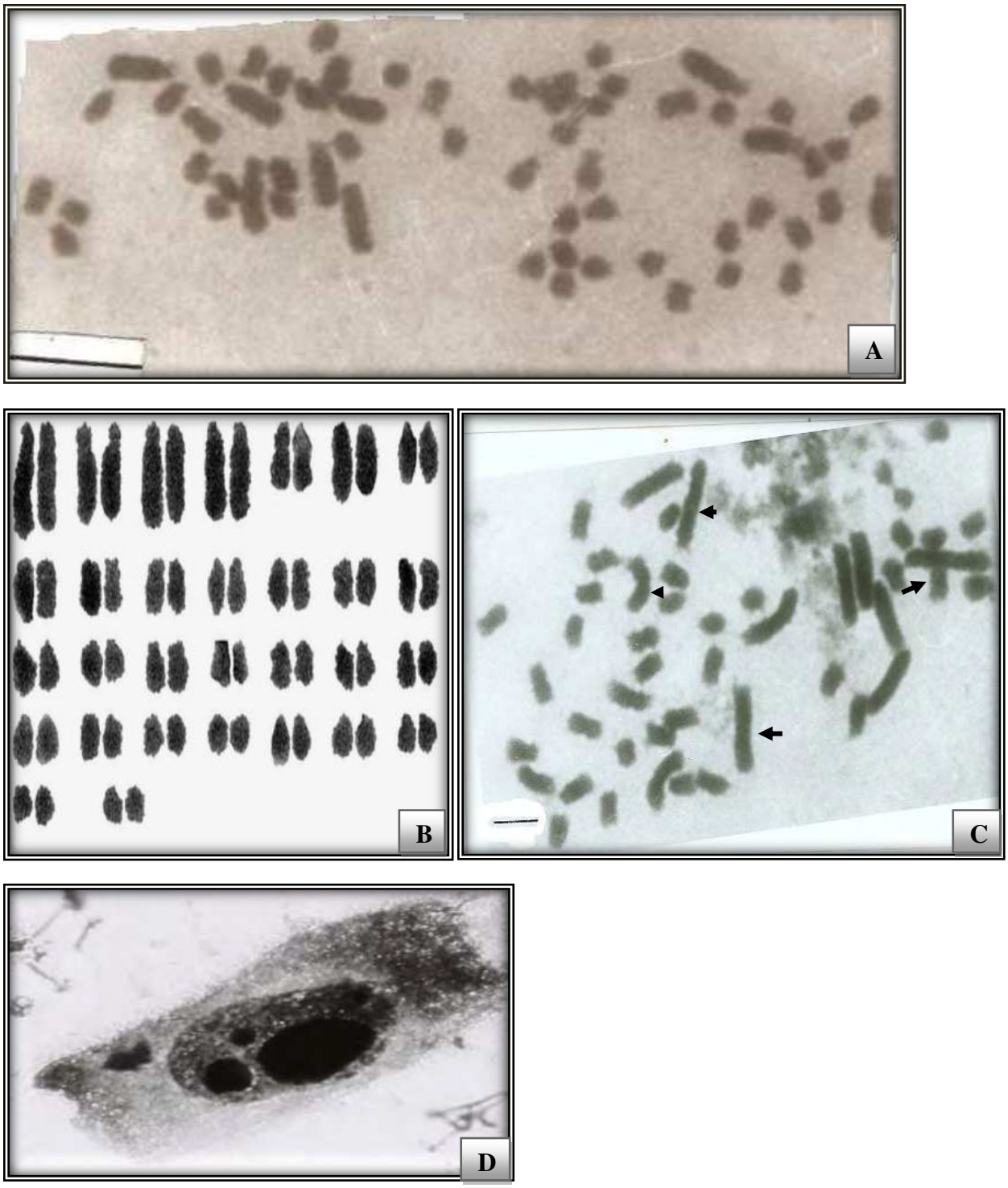

Figure 10. Chromosome spread, karyotype and nucleoli of *Tradescantia fluminensis* (green) (A). chromosome number, (B). karyotype, (C). spread containing some bi-armed chromosomes (Scale bar = 5μm) and (D). three nucleoli. Scale bar = 10μm

Tradescantia fluminensis (green) possess the highest coefficient of variation in chromosome length (32.78) and inter-chromosomal asymmetry index (0.3278). Ratio of centromeric gradient (22.93), intra-chromosomal asymmetry index (0.7252), coefficient of variation in chromosome length (32.78) and dispersion index (7.52) were also calculated (Table 4).

5.6 *Tradescantia fluminensis* (Variegated)

According to the result obtained from metaphase chromosomes spread, this variant also possesses 2*n*=60 (Figure 11 and Table 2). Since good metaphase spread of chromosomes suitable for karyotypic and for making of measurements were not obtained, this report was restricted only on determination of the 2*n* chromosome number.

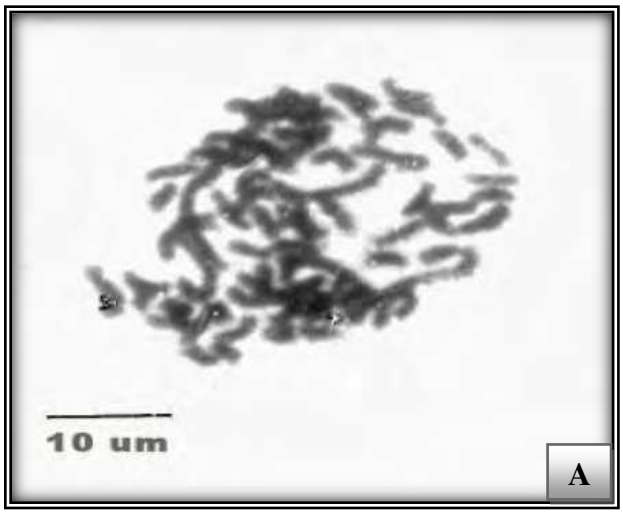

Figure 11. Metaphase chromosomes of *Tradescantia fluminensis* (variegated) from Addis Ababa.

5.7 *Tradescantia zebrina*

This diploid species possesses 2*n*=2*x*= 24 (Table 2 and Figure 10). The karyotype constructed on the bases of arm ratio and chromosome length (Appendix 7), possesses two metacentric pairs (pairs 1and 2), three sub-telocentric pairs (pairs 3 to 5) and seven telocentric chromosome pairs (pairs 6 to 12) and has a formula of 4m + 6st + 14t. The asymmetry index is 77.375 and so it

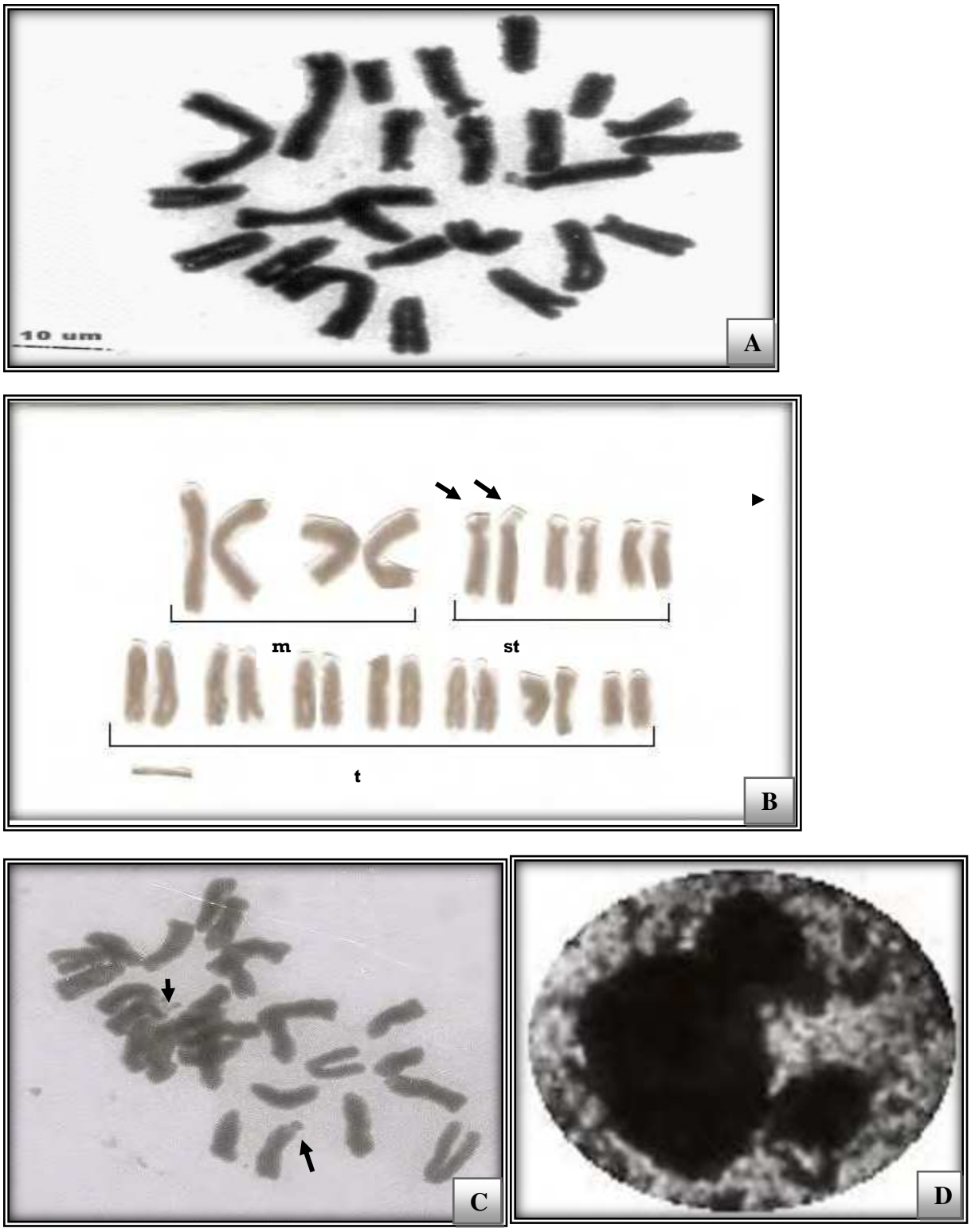

Figure 12. Chromosome number, karyotype and nucleoli of *Tradescantia zebrina* (A). chromosome number, (B). karyotype, (C). three satellites and (D). three nucleoli, two small and one large. Scale bar = 10µm

belongs to 3B type of asymmetry class of Stebbins (Table 3). Two satellites (Figure 12C) were observed and with silver staining the number of interphase nucleoli obtained was three (Figure 12C). The fact that one of the nucleoli is larger than the other two may imply that it is the result of fusion of two or more small nucleoi. Thus, it can be inferred that the number of NORs and so number of satellites may be four or more.

Tradescantia zebrina have the largest chromosome than other species included in this study with size variation ranging from shortest (7.031µm) to longest (19.0625µm) and total length of whole chromosome complement 269.952 µm (Table 3 and Appendix 7). The total length of long arms and short arms for the diploid set was 208.877µm and 61.075µm respectively (Appendix 7). The chromosomes were largest in size with an average length of 11.04µm. Generally the chromosomes were sub-telocentrics and possess an average karyotype asymmetry of 3.42. The karyotype was of bimodal type.

With lowest value in ratio of centromeric gradient (11.987) and dispersion index (3.512), this particular species has inter-chromosomal asymmetry indices (0.2931), intra-chromosomal asymmetry (0.73174) and coefficient of variation in chromosome length of (29.31) (Table 4).

6. Discussion

In the present study, six species belonging to the family Commelinaceae were investigated with regards to the chromosome cytology. In the four species studied from the genus *commelina*, base numbers of *x*=11 and *x*=15 have been obtained. Base numbers *x*=11, 13 and 15 was also previously reported for *Commelina* (Jones and Jopings, 1972; Faden and Suda, 1980). The species *Commelina benghalensis* (2*n*=6*x*=66) was based on *x*=11. Others, *Commelina africana* (Addis Ababa and Sebeta), *Commelina diffusa* (Entoto and Jimma) and *C. subulata* having chromosome number of (2*n*=2*x*=30) were based on *x*=15 base number. This result agrees with previous studies (Lewis and Tadesse Eba, 1964; Fukumoto, 1964; Bhattacharva, 1975; Faden and Suda, 1980; Alam and Sharma, 1984; Eksomtramage *et al.,* 2001; Fujishima 2007a; Fujishima, 2007b).

The chromosome numbers for *T. zebrina* (2*n*=2*x*=24) was based on *x*=12 and this is in agreement with the findings of Lewis and Tadesse Eba (1964). *T. fluminensis* (green) possessing 2*n*=60 was also in agreement with the findings of Darlington (1929) and Weryszko-Chmielewska (1989). Also Martinez (1984) reported chromosome number 2*n*= 40 for *T. fluminensis*, but it does not match with the current chromosome count. *Commelina subulata* of Ginchi has 2*n*=30 and this was in agreement with other reports from materials collected from Ethiopia (Lewis and Taddese Eba, 1964) and Ghana (Morton, 1967). Tetraploid species of *Commelina subulata* 2*n*=60 have also been reported for materials from India (Kammathy and Rao, 1961; Raghaven and Rao, 1961). *Commelina diffusa* from Ginchi has 2*n*=66 and this number is reported for the first time.

Chromosome numbers of *Commelina africana* collected from Addis Ababa and Sebeta were diploids 2*n*=30 and this was in agreement with previous reports (Lewis, 1964; Lewis and Taddese Eba, 1964; Morton, 1967). Even if, the current count was diploid chromosome number,

Morton (1956) also found 2*n*=28 for materials from Ghana and polyploids (2*n*=60 and 2*n*= 120) were also reported by Lewis (1964). *C. benghalensis* of Addis Ababa was found to be hexaploids (2*n*=6*x*=66) and this was in agreement with earlier report (Morton, 1967). Both diploid 2*n*=22 and tetraploid (2*n*=44) cytotypes have also been reported for *C. benghalensis* from materials of China, India, Japan, Uganda, Ethiopia and Tanganyika (Fukumoto, 1964; Lewis and Tadesse, 1964; Lewis, 1964; Morton, 1967; Bhattacharya, 1975; Alam and Sharma, 1984; Shigenobu and Kobori, 1997; Fujishima, 2007a. A diploid *C. diffusa* (2*n*=30) has been found for specimens collected from Entoto mountain and Jimma. This result was in agreement with other reports (Lewis, 1964; Lewis and Tadesse, 1964; Panigraphi and Kammathy, 1964; Fujushima, 2007b). However, Morton (1956) also found 2*n*=28. *T. zebrina* (2*n*=2*x*=24) was also found to be diploid and this was in agreement with reports of Lewis and Taddese Eba (1967) from Ethiopian material.

Morton (1967) suggested that polyploid series of the West African *Commlina benghalensis*, *Commelina africana* and *Aneilema umbrsum* complexes are of autopolyploid origin based on observation of close similarity between polyploid and diploids with the absence of allied taxa which could have been involved in allopolyploidy.

A comparison made between the karyotypes of *C. diffusa* described by Fujishima (2007a) from Taiwan and *C. diffusa* (Entoto mountain and Jimma) in the present study indicates that, although they share similar chromosome number (2*n*=30), they differ in karyotypic detail. The karyotype formula of the Taiwan specimen was reported with only two chromosomal groups, m and sm, (10m + 20sm), while in the present materials three chromosomal groups (m, sm and st) with a formula of 16m+6sm+8st (Entoto) and 10m + 8sm + 12st (Jimma) were observed. Alam and Sharma (1984) reported variation in karyotypes among five populations of Indian *C. diffusa*

having 2*n* = 30. Bhattacharya (1975) also reported four different karyotype formulae within 2*n*=30 chromosomes from India. Factors, other than the chromosomal heteromorphism, like deviation in techniques of chromosome preparation, condensation difference and measurement technique can also lead to karyotype diversity among reports by different workers. These factors hinder comparison between various karyotypes and for real comparison mean values for each measurement of individual chromosomes must be taken. Furthermore, karyotype comparison for the remaining species was not performed as all the chromosome report matching to the current chromosome number had not been found.

The chromosome report 2*n*=28 for the two species of *C. diffusa* and *C. africana* by Morton (1956) was probably associated with the uncommon aneuploids formed due to loss of chromosome number (Lewis and Taddesse Eba, 1964). But reduction in chromosome number can be associated with Robertsonian fusion (Myrose *et al.,* 2010) and translocation of all or most of its part followed by loss of the chromosome. Morton (1967) also indicated high rate of prevalence of aneuploidy and polyploidy in the genus *Commelina*.

Jones and Joplings (1972) reported the chromosome size of the genus *Commelina* as the smallest in the family. Faden (1980) and Morton (1967), on the other hand, confirmed the presence of medium to relatively large chromosome within the genus. In the present study, except for *Commelina subulata*, the size was predominantly medium, whereas *C. subulata* has an average chromosome smaller than the medium size limit (3µm).

The karyotype data of the present materials in the genus *Commelina* indicated the presence of three types of chromosomes with higher frequency of metacentric (m) and sub-metacentric (sm) types than sub-telocentrics (st). This was supported by earlier reports (Morton, 1967; Faden and

Suda, 1980). However the ratio of each chromosome type considerably varies between each species.

Species studied under genus *Tradescantia* (*T. fluminensis* and *T. zebrina*) have bimodal karyotypes that have differentiated from that of the other species of genus *Commelina*. According to Jackson (1971), species with bimodal karyotypes tend to possess more asymmetric karyotypes that performed several breakage and reunion of chromosomal segment. Moreover, asymmetric karyotypes have an evolutionary advantage as it might carryout mitosis more rapidly than symmetric karyotypes due to possession of small metacentric or acrocentric chromosomes that could possibly separated easily during the anaphase stage (Stebbins, 1971).

The comparisons made between chromosomes of *T. zebrina* from materials of China (Zhang, 1989) and the current material from Addis Ababa show similarity with regard to chromosome number. But variation was observed in terms of karyotype formula, Stebbins chromosome asymmetry type and ratio of largest to smallest chromosomes. *T. zebrina* of Addis ababa possesses a karyotype formula of 4m +6st + 14t, ratio of smallest to largest chromosome (2.71) with 3B type of Stebbin's asymmetry, while *T. zebrina* (Zhang, 1989) has a karyotype formula (4m + 6sm + 14t), ratio of smallest to largest chromosome (2.58) and has a asymmetry of 2B type. The slight karyotype asymmetry difference between the African and Asian species might be associated with chromosomal rearrangement such as unequal translocation and deletion which is triggered by environmental variation.

A comparison made based on asymmetric index in the current study indicates that *C. africana* collected from Addis Ababa has same karyotype asymmetry as *C. africana* of Sebeta with a value of 65.19% and 65.89%, respectively. *C. diffusa* (Entoto) is of similar karyotype asymmetry as *C. diffusa* (Jimma) with asymmetric index of 66.76% and 69.497%, respectively. Similarly *C.*

diffusa (Ginchi) has karyotype of about same symmetry as the above two variants and possesses an asymmetric index of 65.56%.

The present study revealed some degree of variation in chromosome length between the studied species with no major chromosomal difference among themselves. For instance, there was slight difference in total chromosome length between *C. africana* (2*n*=2*x*=30) of Addis Ababa and *C. africana* (2*n*=2*x*=30) of Sebeta. Likewise *C. diffusa* (2*n*=2*x*=30) collected from Entoto mountain is also somewhat greater in chromosome length than *C. diffusa* (2*n*=2*x*=30) of Jimma. This may be associated with differences in degree of chromosome condensation between the metaphase spread of different species measured. Thus, in practice, it is difficult to draw taxonomic conclusions simply by comparing chromosome length between taxa (Carter *et al.,* 1984) unless one compares chromosomes condensed to same degree.

Satellited chromosomes were observed frequently in Commelinaceae (Morton, 1967). In the present study, satellites were observed in about half of the studied species like *C. africana* (A.A), *C. africana* (Sebeta), *C. diffusa* (Entoto), *C. diffusa* (Ginchi) and *T. zebrina*. The reason why satellites were not observed in some of the species may be that the satellites are too small and escape easy cytological detection, or condense. All the satellites detected were located at the tip of the short arm of chromosome. Discrepancies in number of satellites reported for a particular taxon or population can be due to the inability to observe all the satellites because of variation in techniques of chromosome preparation, stages at the time of chromosome analysis and chromosomal polymorphism (Kifle Dagne, 1995). From the current investigation, two satellites were observed in *C. africana* (A.A), four in *C. africana* (Sebeta) and *C. diffusa* (Entoto mountain), six in *C. diffusa* (Ginchi) and two in *T. zebrina*.

The maximum number of telophase nucleoli obtained through silver staining may correspond to the maximum number of satellites that are present in particular taxon (Kifle Dagne, 1995). Thus, even though all the satellites are not detected for various reasons discussed above, one can infer about the number of satellited chromosomes present in the taxon, if one is able to obtain the maximum number of nucleoli present. Accordingly it was assumed that the maximum number of satellites for *C. africana* (A.A) will be four. Equal correspondence between number of nucleoli and satellites was obtained for *C. diffusa* (Entoto) and *C. africana* (Sebeta).

Nucleoli are formed at the nucleolus organizer regions (NORs) of the satellited chromosomes. During the cell cycle, nucleoli disappear at late prophase and reform during telophase. It is less easy to observe the maximum number of nucleoli during telophase. As the cell cycle proceeds from telophase to interphase, nucleoli tend to fuse together, and thus their number in most of the interphase nuclei is usually less than their number in telophase nuclei. Though it is less frequently, it is possible that the nucleus enters interphase without all the nucleoli being fused, in which case the maximum number of nucleoli can also be observed in interphase nuclei. The maximum number of nucleoli observed, be at telophase or interphase, can be used to infer about the number of active NORs the plant possesses. Even numbers of maximum nucleoli are expected because NORs chromosomes occur as homologous pair (s). In case the highest number observed is odd number, one may take the next higher even number as the number of nucleoli for the organism. Usually when odd number is observed, at least one of the nucleoli is larger than the rest of the nucleoli indicating that the large nucleolus is the product of fusion of smaller nucleoli. In the present study, maximum number of nucleoli observed for *C. Africana* (Addis Ababa) is three, and for *C. africana* (sebeta) and *C. diffusa* (Entoto) is four each which allow to make an inference that they all have 4 NORs (satellite chromosomes).

The number of nucleoli observed for *C. benghalensis* is five but two are very large relative to the other three nucleoli. The large ones are possibly fusion products of two or more small nucleoli. There are at least three pairs or more satellited chromosomes in this species.

In *T. zebrina*, there are one large and two small nucleoli, the former being fusion product of two or more nucleoli, implying that there are at least four NORs.

In *T. fluminensis*, the nucleoli observed were one very small, one small and one very large. The large nucleolus could be result of fusion of several small nucleoli. Though not possible to infer the number of NORs, it seems that more than two pairs of NORs are present.

7. Conclusion and Recommendations

7.1 Conclusion

The present study has revealed and confirmed chromosome number, ploidy level, karyotype and nucleolus numbers of six species of Commelinaceae which were collected from different localities of central and south western part of the country. Accordingly, this study showed that basic chromosome number for three species of *Commelina* (*C. diffusa, C. africana* and *C. subulata*) is x=15 and $2n$=30. The chromosomes of these species are predominantly of m and sm types. *C. benghalensis* ($2n$=6x=66) was also found to have x=11. *T. zebrina* was also known to have $2n$=24 and a basic chromosome number x=12. This was in common with other reports. The basic chromosome number for *C. diffusa* (Ginchi), $2n$=66 was different from previous reports and hence it could be another cytotype for the species. Variation in karyotype formula is also observed within species of *C. africana* and *C. diffusa* collected from different localities. Different karyotype formula is also prevailed among the six species. *C. diffusa* (Ginchi) is vigour, longer with distinct morphology than the other diploid species collected from Jimma and Entoto Mountain.

This is the first work to present karyotypes, satellite chromosomes and nucleoli of the Ethiopian Commelinaceae. The study also includes the first chromosome report for *C. diffusa* ($2n$=66). Because of chromosome overlapping, detailed chromosome analysis was not possible for *T. fluminensis* (variegated) and thus only chromosome number were reported. Generally, this study provides additional chromosomal information for Ethiopian material in addition to the earlier meiotic chromosome report of Lewis and Taddese Eba (1964). However, it is not complete enough to reveal clues about evolution and phylogenetic relationship within and among species of the family.

7.2 Recommendations

Based on the chromosome analysis of the six species of Commelinaceae collected from different locations, the following points require further consideration:

- Detail chromosome research with regard to number, morphology and structural rearrangements is required for species' found under the genus *Commelina* covering diversified geographical distribution throughout the country, for better confirmation and understanding of their karyotypes.
- As most of the species in the family Commelinaceae reproduced vegetatively, any adaptable karyotypes could be maintained in the region where they reproduced and distributed to adjacent sites. Therefore, further analysis with regard to the relationship between cytological characters and geographic distribution is required especially for vegetatively reproduced species.
- An integrated data from karyotype, molecular study of chloroplast genome and evolution through considering more representative species is necessary to infer phylogenetic relationship among the taxa.
- Since polyploidy seem to be common among the species of *Tradescantia* and *Commelina*, meiosis study covering wider species is demanding to show the behavior of chromosomes at various stages and possibly confirm their nature of polyploidy.
- For better differentiation of closely related species having similar karyotypes, C-banding and silver staining are recommended as these techniques help identify the homologoues chromosomes by revealing variation in location and size of NORs and heterochromatin along the lengths of chromosomes

8. References

Alam, N. and Sharma, A.K. (1984). Trends of chromosome evolution in family Commelinaceae. *The Nucleus* **27:** 231-241.

Arabbeigi, M., Arzani, A. and Saeidi, G. (2011). Study of karyotype and nucleolar organizer region (NORs) in wild, synthetic and cultivated wheats. *Emir. J. Food. Agric.* **23(2):** 196-203.

Avramova, Z. V. (2002). Heterochromatin in animals and plants. Similarities and differences. *Plant Physiol.* **129:** 40-49.

Barcia, J.J. (2007). The Giemsa stain: its history and applications . *Int. J. Surg. Pathol.* **15:** 292-296

Bennett, M.D. and Smith, J.B. (1976). Nuclear DNA amounts in angiosperms. *Phil. Trans. Roy. Soc. Lond* **274:** 227–274.

Bennett, M.D., Smith, J.B. and Seal, A.G. (1986). The karyotype of the grass *Zingeria biebersteiniana* (2*n* = 4) by light and electron microscopy. *Can. J. Genet Cytol.* **28:** 554-562.

Bhattacharya, B. (1975)**.** Cytological studies on some members of Indian Commelinaceae. *Cytologia* **40:** 285–299.

Briggs, B.G. (1966). Chromosome numbers of some Australian monocotyledons. *Contribution from the New South Wales National Herbarium* **4:**24–34.

Carter, S., Cutler, D.F. and Brandham, P.E. (1984). A multidisciplinary approach to revision of the *Aloe somaliensis* complex (Liliaceae). *Kew Bulletin* **39:** 611-633.

Chen, Z. J. (2010). Molecular mechanisms of polploidy and hybrid vigour. *Trends Plant Sci*. **15:** 57-71.

Celarier, R.P. (1955). Cytology of the Tradescantieae. *Bull. Torrey. Bot. Club* **82:**30-38.

Chaudhary, S.A (2002). *Chromosome Banding.* Seminar Report, Karnataka.

Coghlan, A., Eichler, E., Oliver, S.G. and Paterson, A.H. (2005). Chromosome evolution in eukaryotes: a multi-kingdom perspective. *Trends. Genet.* **21(12):** 673-682.

Crosland, M.W and Crozier, V. (1986). *Myrmecia pilosula,* an ant with only one pair of chromosomes. *Science* **231:** 1278.

Darlington, C.D. (1929). Chromosome behavior and structural hybridity in the Tradescantiae. *J. Genet.* **21:** 207-284.

De Bodt, S., Maere, S. and Van de Peer, Y. (2005). Genome duplication and the origin of angiosperms. *Trends. Ecol. Evol.* **20:** 591-597.

Dietrich, A. J. (1986). The influence of fixation on the morphology of mitotic chromosomes. *Can. J. Genet. Cytol.* **28:** 536-539.

Drets, M.E. (2004). Cytological indications of the complex subtelomeric structure. *Cytogenic Genome Res.* **104:** 137-141.

Eksomtramage, L., Sirirugsa, P., Sawangchote, P., Jornead, S., Saknimit, T. and Leeratiwong, C. (2001). Chromosome numbers of some monocot species from Ton-NGA-Chang wildlife sanctuary, Southern Thailand. *Thai. For. Bull. (Bot)* **29:** 63-71.

Ensermu Kelbessa and Faden, B., (1997). Commelinaceae. **In:** *Flora of Ethiopia and Eritrea*, (Edwards, S., Mesfin Tadesse, Demissew Sebsebe & Hedberg, I. ,eds). Hydrocharitaceae to Arecaceae. The National Herbarium, Addis Ababa University, Addis Ababa, and Department of Systematic Botany, Uppsala University, Uppsala.

Faden, R.B. (1983). Phytogeography of African Commelinaceae. *Bothalia* **14:** 553–557.

Faden, R. B. (1994). New species of *Commelina* (Commelinaceae) from the Flora of Tropical East Africa. *Novon* **4:** 22-235.

Faden, R. B. (1998). Commelinaceae. **In:** *The Families and Genera of Vascular Plants,* pp. 109-128, *(*Kubitzki, ed). Springer-Verlag, Berlin.

Faden, R. B. (1980). Floral Biology of Commelinaceae. In: *Monocots: Systematics and Evolution*, pp. 309–318, (Wilson, K.L. and Morrison, D.A., eds) . CSIRO, Melbourne.

Faden, R. B. (2007). New species *Tricarpelema* (Commelinacea) from Africa and Asia, including the first record of the genus from Africa and a synopsis of the genus. *Novon* **17(2):** 160-171.

Faden, R. B. and Alford, M.H., (2001). A new species of *Commelina* (Commelinaceae) from Tanzania. *Novon* **11:**16-21.

Faden, R. B. and Hunt, D.R. (1991). The classification of the Commelinaceae. *Taxon* **40:** 19-31.

Faden, R.B. and Suda, Y. (1980)**.** Cytotaxonomy of Commelinaceae: Chromosome numbers of some African and Asiatic species. *J. Linn. Soc. Bot*. **81:** 301–325.

Fatkhutdinova, R. A., Shakirova, F. M., Chemeris, A. V., Sabirzhanov, B. E. and Vakhitov, A. V. (2002). NOR activity in wheat species with different ploidy levels treated with phytohormones. *Rus. J. Genet.* **38:**1335-1338.

Fukumoto, K. (1964). Examination of the chromosome numbers and karyotypes of some *Commelina* species. *J. Japan. Gen* **39:** 340-347.

Fujishima, H. (2007a). Karyotype diversity of *Commelina benghalensis* L. (Commelinaceae). *Chromosome Science* **10:** 53-63.

Fujishima, H. (2007b). Karyotype diversity of *Commelina diffusa* Burm. (Commelinacea). *Chromosome Science* **10:** 21-28.

Geesing, D. and Djibo, H. (2001). Niger-Country Pasture/forage resource profiles. **In**: *Grassland and Pasture Crops*. FAO, Technical University of Munich, Munich.

Glimn-Lacy, J. and Kaufman, P.B. (2006). *Introduction to Plants, Major Groups, Flowering Plant Families*. Springer, London.

Grant, V. (1975). *Genetics of Fowering Plants.* Colombia University Press, New York.

Gregory, T.R. (2004). Insertion-deletion biases and the evolution of genome size. *Gene* **324:** 15-34.

Greilhuber, J. (1995). Chromosomes of the monocotyledons. **In:** *Monocots: Systematic and Evolution,* (Rudall, P., Cribb, P., Cutler, D. and Humphries, C. eds). RBG, London.

Greilhuber, J., and Ehrendorfer, E. (1988). Karyological approach to plant taxonomy. *ISI Atlas Sci.: Anim. Plant Sci.* **1:** 289-297.

Greilhuber, J., Dolezel, J., Lysak, M. and Bennett, M.D. (2005). The origin, evolution and proposed stabilization of the term genome size and c-value to describe nuclear DNA contents. *Ann. Bot.* **95:** 255-260.

Grimn-lacy, J. and Kaufman, P.B (2006). *Botany Illustrated: An Introduction to Plants, Major Groups, Flowering Plant Families*. Springer, New York.

Guerra, M. (2008). Chromosome numbers in plant cytotaxonomy: concepts and implications. *Cytogenet. Genome Res.* **120:** 339-350.

Guerra, M. (2000). Chromosome number variation and evolution in monocots. **In:** *Monocots: Systematics and Evolution* (Wilson, K. And Morrison, D., eds). CSIRO, Melbourne.

Guerra, M. (2005). What is new in plant cytogenetics? *Genet. Mol. Biol.* **28:** 444-445.

Gupta, P.K (2006). Plant cytogenetics: a rebirth in twenty-first century. *Indian J. Crop Science* **1:** 1-7.

Hasan, S.M., Hossain, M., Akter, R., Jamila, M. and Mazumber, M.E. (2010). Analgesic activity of the different fractions of the aerial parts of *Commelina benghalensis* Linn. *Inter. J. Pharma* **6(1):** 63-67.

Heslop-Harrison, J.S. and Schwarzacher, T. (2011). The plant genome: an evolutionary view on structure and function. *Plant Journal* **66:** 18-33.

Hizumem, M., Sato, S. and Tanaka, A. (1980). A highly reproducible method of nucleolus organizing regions staining in plants. *Stain Technol*. **55:** 87-90.

Hoffman, A. and Rieseberg, L.H. (2008). Revisiting the impact of inversions in evolution: from population genetic markers to drivers of adaptive shifts and speciation? *Ann. Rev. Ecol. Evol. Syst.* **39:** 21-42.

Hong, D.Y., Deyuan , D. and Robert, A. (2000). *Commelina diffusa*. **In**: *Flora of China*, pp.36-48, (Wu, Z. Y., Raven, P.H. and Hong, D.Y., eds). Beijing Science Press and St. Louis: Missouri Botanical Garden Press, Beijing.

Ivens, G.W. (1967). *East African Weeds and their Control*. Oxford University Press, Nairobi.

Jackson, B.C. (1971). The karyotype in systematics. *Ann. Rev. Ecol. Syst.* **1971(2):** 327-368.

Jayvir, A., Minoo, P., Gauri, B. and Ripal, K. (2002). *Nature Heals: A Glossary of Selected Indigenous Medicinal Plants of India*. SRIST Innovations, Ahmedabad.

Jemilat, I., Chioma, A.V. and Omoregie, H. (2010). Pharmacognostic and phytochemical analysis of *Commelina benghalensis* L. *Ethnobotanical Leaflet* **14:** 610-615.

Johnson, M., Kenton, A.Y., Bennet, M. and Brandham, P.E (1989). *Voaniola gerandii* has the highest known chromosome number in the monocotyledons. *Genome* **32:** 328-333.

Jones, K. and Jopling, C. (1972)**.** Chromosomes and the classification of the Commelinaceae. *J. Linn. Soc. Bot.* **65:**129–162.

Jones, N., and Houben, A. (2003). B chromosomes in plants: escapes from the A chromosome genome? *Trend. Pl. Sci.* **8:** 417-423.

Jones, R. N., Viegas, W. and Houben, A. (2008). A century of B chromosomes in plants: so what? *Ann. Bot.* **101:** 767-775.

Kammathy , R.V and Rao, R.S. (1961). Notes on Indian Commelinaceae.II. Cytological observation. *Bull. Bot. Sur.Ind.* **3:**167–169.

Kato, A., Lamb, J.C. and Birchler, J.A. (2004).Chromosome painting using repetitive DNA sequences as probes for somatic chromosome identification in maize. *Proc. Natl. Acad. Sci.* **101:** 13554-13559.

Kifle Dagne and Heneen, W.K. (1992). The Karyotype and nucleoli of *Guizotia abyssinica* (Compositae). *Hereditas* **117:** 73-83.

Kifle Dagne (1995). Karyotypes, C-banding and nucleolar numbers in *Guizotia* (Compositae). *Pl. Syst. Evol.* **195:** 121-135.

Kubitzia, K. (1998). *The Families and Genera of Vascular Plants*. Springer, New York.

Kumari, G. and Roy, B. K. (2010). Karyotype studies in dominant species of *Aloe* from eastern India. *Caryologia* **63(1):** 41-49.

Kunle, O. F. and Egharevba, H. O. (2009). Preliminary studies on *Vernonia ambigua*: phytochemistry and antimicrobial screening of whole plant. *Ethnobotanical Leaflets* **13:** 1216-122.

Levan, A., Fredga, K., and Sanderberg, A.A. (1964). Nomenclature for centromeric position on chromosomes. *Hereditas* **52(2):** 201-220.

Levin, D. A. (2002). *The Role of Chromosomal Change in Plant Evolution*. Oxford University Press, New York.

Lewis, W. H. (1964). Meiotic chromosomes in African Commelinaceae. *Sida* **1:** 274-293.

Lewis, W. H. and Taddesse Eba (1964). Chromosome numbers in Ethiopian Commelinaceae. *Kirkia* **4:**213-215.

Li, X., Zhu, C., Lin, Z., Wu, Y., Zhang, D., Bai,G., Song, W., Ma, J., Muehlbauer, G., Scanlon, M., Zhang, M. and Yu, J. (2011). *Chromosome Size in Diploid Eukaryotic Species Centers on the Average Length with a Conserved Boundary*. Oxford University Press, England.

Lomax, B. H., Woodward, F., Leitch, I., Knight, C. and Lake, J. (2009). Genome size as a predictor of guard cell length in *Arabidopsis thaliana* is independent of environmental conditions. *New Phytol.* **181**(2): 311-314.

Lynch, M. 2007. *The Origins of Genome Architecture.* Sinauer Associates, Sunderland.

Mao, X., Nie, W., Wang, J., Su, W., Feng, Q., Wang, Y., Dobigny, G. and Yang, F. (2008). Comparative cytogenetics of bats (Chiroptera): the prevalence of Robertsonian

translocations limits the power of chromosomal characters in resolving interfamily phylogelogenetic relationships. *Chromosome Res*. **16:** 132-144.

Mabberley, D. (1997). *The Plant-Book.* Cambridge University Press, Cambridge.

Malik, C.P. (1961)**.** Chromosome number in some Indian angiosperms: monocotyledons. *Science and Culture,* **27:**197–198.

Manandhar, N. P., Manandhar, P. and Sanjay (2002). *Plants and People of Nepal*. Timber Press, Portland.

Morton, J.K. (1956)**.** Cytotaxonomic studies on the Gold Coast species of the genus *Commelina J*. *Linn*. *Soc*. *Bot*. **55:**507–531.

Morton, J.K. (1967)**.** The Commelinaceae of West Africa: a biosystematic survey. *J*. *Linn*. *Soc*. *Bot*. **60:** 167–221.

Mudau, R.(2007). *Commelina africana L* . Pretora National Herbarium, South Africa.

NAPPO, (2003). *Commelina benghalensis* Plant Pest Fact Sheet, California.

Panigraphi, G. and Kammathy, R.V. (1964). Cytotaxonomic studies in certain species of Commelina Linn. in eastern India. *J. Indian Bot. Soc.* **43:** 294-310.

Poole, M. M. and Hunt, D. R. (1980). Pollen morphology and the taxonomy of the Commelinacea: an exploratory survey. *Kew Bull*. **34:** 639-660.

Qureshi, R. and Bhatti, G.R. (2008). Ethnobotany of plants used by the Thari people of Nara Desert, Pakistan. *Fitoterapia* **79:** 468-473.

Raghaven, R.S. and Rao, R.S (1961). Cytological observations on the Indian species of Commelinaceae. *Curr. Sc.* **30:** 310-311.

Romero Zarco, C. (1986). A new method for estimating karyotype asymmetry. *Taxon* **35:** 526-530.

Saez-vasquez, J. and Medina, F. (2008). Plant nucleolus. *Adv. Bot. Res.* **47:** 1-35.

Sakurai, T. And Echikawa, S. (2001). Karyotypes and Giemsa C-banding patterns of *Zebrina pendula*, *Zebrina purpusii* and *Setcreasea purpurea*, compared with those of *Tradescantia ohiensis*. *Genes and Genetic Systems.* **76:** 235-242.

Savage, J.R. (2004). On the nature of visible chromosomal gaps and breaks. *Cytogenet Genome Res.* **104:** 46-55.

Schubert, I. (2007). Chromosome evolution. *Curr. Opin. Plant Biol.* **10**:109-115.

Sharma, A.K. (1955)**.** Cytology of some of the members of Commelinaceae and its bearing on the interpretation of phylogeny. *Genetica* **27:** 323–363.

Shigenobu, Y. and Kobori, K. (1997). Karyotype variation of *Commelina benghalensis* correlated with habitats in Kochi preference, Japan. *Chromosome Science* **1:** 83-87.

Smith, A. R., Pryer, K. M., Schuettpelz, E., Korall, P., Schneider, H. and Wolf, P. G. (2006). A classification for extant ferns. *Taxon* **55:** 705–731.

Stace, C. A. (2000). Cytology and cytogenetics as a fundamental taxonomic resource for the 20th and 21th centuries. *Taxon* **49:** 451-477.

Stebbins, G. L. (1971). *Chromosomal Evolution in Higher Plants*. Edward Arnold (Publishers) Ltd, London.

Stindle, R. (2004). Is telomere erosion a mechanism of species extinction? *J. Exp. Zool*. **302:** 111-120.

Sumner, A. T. (2003). *Chromosomes: Organization and Function.* Blackwell Science Ltd, Oxford.

Techio, V.H., Davide, L.C., Cagliari, A., Barbosa, S. and Pereira, A.V. (2010). Karyotipic asymmetry of both wild and cultivated species of *Pennisetum*. *Bragantia* **69:** 273-279.

Tezera Temesgen (2008). *Chromosomes Study of Eleven Endemic Aloe Species of Ethiopia with Emphasis on the Satellites and Nucleoli*. Master's Thesis. Addis Ababa University, Addis Ababa.

Uhl, C.H. (1992). Polyploid, dysploidy, and chromosome pairing in *Echeveria* (Crassulaceae) and its hybrids. *Amer J Bot* **79:**556–566.

Van der Burg, W.J. (2004a). *Commelina africana L.* (Grubben, G.J.H. and Denton, O.A., eds). PROTA, Wageningen.

Van der Burg, W.J. (2004b). *Commelina benghalensis L.* (Grubben, G.J. and Denton, O.A., eds). PROTA, Wageningen.

Verma, P.S. and Agarwal, V.K. (2005). *Cell Biology, Genetics, Molecular Biology, Evolution and Ecology.* S. Chand and Company Ltd, New Delhi.

Weryszko-Chmielewska, E. (1989). An investigation of the karyotypes of four species from the genera *Setcreasea*, *Spironema* and *Tradescanta* (Commelinaceae). *Acta. Soc. Bot. Poloniae* **58:** 527-540.

Whitney, K.D., Baack, E.J., Hamrick, J.L., Godt, M., Barringer, B.C., Bennet, M., Eckert, C., Goodwillie, C., Kalisz, S., Leitch, I. and Ross-Ibbara, J. (2010). A role of maladaptive processes in plant genome size evolution. *Evolution* **64:** 2097-2109.

Zakian, V.A. (1989) Structure and function of telomeres. *Ann Rev Genet* **23:** 579-604.

Zhang, D. Z. (1989). Karyotype analysis of *Zebrina pendula* Schnizl. *J. Anhui Normal Univ*. *Nat. Sci.***04:** 110-115.

Zheng, J.Y., Gu, C.Y. and Chen, R.Y. (1989)**.** Cytotaxonomical studies on Commelinaceae in China. Chromosome numbers and karyotypes of some Chinese species. **In:** *Plantchromosome research,* pp. 363–368, (Hong D. , ed) . Organizing Committee of the Sino-Japanese Symposium on Plant Chromosomes, Beijing.

Zurita, F., Jimenez, R., Burgos, M. and Diaz de la Guardia, R. (1998). Sequential silver staining and *in situ* hybridization revieal a direct association between rDNA levels and the expression of homologous nuclear organization regions: a hypothesis for NOR structure and function. *J. Cell Sci.* **111:** 1433-1439.

Appendix 1: Chromosome measurements (chromosome length, arm length, arm ratio and centromere position) of *Commelina africana* from Addis Ababa

Image magnification: 2200

Image resolution: 78.4 pixels per centimeter

Chromosome rank	Length each	Long arm	Short arm	Arm Ratio (L/S)	Cent. Index (S/(L+S))	Chromosome form
1	5.323651	3.111069	2.212582	1.406081	0.415614	m
2	5.012773	3.358625	1.654148	2.030426	0.329987	sm
3	4.9391	3.317681	1.621419	2.04616	0.328282	sm
4	4.877695	2.705329	2.172367	1.245337	0.445367	m
5	4.7457	2.765829	1.979871	1.396974	0.417193	m
6	4.694626	2.409005	2.285621	1.053983	0.486859	m
7	4.650337	2.8344	1.815936	1.560848	0.390496	m
8	4.619032	3.025835	1.593197	1.899223	0.34492	sm
9	4.559304	2.644937	1.914367	1.381625	0.419881	m
10	4.474445	2.373898	2.100547	1.130133	0.469454	m
11	4.375935	2.960731	1.415204	2.092087	0.323406	sm
12	4.253113	3.265363	0.98775	3.305859	0.232242	st
13	4.215533	3.389002	0.82653	4.100275	0.196068	st
14	4.208267	3.312096	0.89617	3.695836	0.212955	st
15	4.148444	3.274837	0.873608	3.748636	0.210587	st
16	4.093332	3.440221	0.653111	5.267437	0.159555	st
17	4.000828	3.226334	0.774494	4.16573	0.193583	st
18	3.962882	2.015623	1.947258	1.035109	0.491374	m
19	3.907304	2.04352	1.863784	1.096436	0.477	m
20	3.776453	2.139522	1.636931	1.307032	0.433457	m
21	3.747826	2.386838	1.360988	1.753754	0.363141	sm
22	3.691089	2.723525	0.967564	2.814826	0.262135	sm
23	3.54464	2.330507	1.214133	1.919482	0.342526	sm
24	3.474101	2.663859	0.810243	3.28773	0.233224	st
25	3.336081	2.50955	0.82653	3.036247	0.247755	st
26	3.316519	1.681697	1.634822	1.028673	0.492933	m
27	3.27068	2.114043	1.156638	1.827749	0.353638	sm
28	3.236042	1.681047	1.554995	1.081063	0.480524	m
29	3.231515	2.096603	1.134912	1.847371	0.351201	sm
30	3.148761	2.269484	0.879278	2.581078	0.279246	sm
Totals for set:	122.836	80.07101	42.765			
Average per set	4.094534					

Appendix 2: Chromosome measurements (chromosome length, arm length, arm ratio and centromere position) of *Commelina benghalensis*

Image magnification: 2200

Image resolution: 78.4 pixels per centimeter

Chromosome number	Length each	Long arm	Short arm	Arm Ratio (L/S)	Cent. Index (S/(L+S))	Chromosome form
1	6.164795	3.518678	2.646116	1.329752	0.42923	m
2	6.159969	4.987877	1.172093	4.255529	0.190276	st
3	5.945159	3.165014	2.780146	1.138434	0.467632	m
4	5.889279	3.382542	2.506737	1.34938	0.425644	m
5	5.886208	3.041668	2.844538	1.069301	0.483255	m
6	5.757722	3.256102	2.501619	1.301598	0.434481	m
7	5.594989	3.290027	2.304962	1.427367	0.411969	m
8	5.572736	3.402355	2.170381	1.56763	0.389464	m
9	5.406959	3.610642	1.796317	2.010025	0.332223	sm
10	5.283497	3.656378	1.627118	2.24715	0.307962	sm
11	5.274768	3.175837	2.098931	1.513074	0.397919	m
12	5.268043	3.422153	1.84589	1.853931	0.350394	sm
13	5.256231	3.212689	2.043543	1.572117	0.388785	m
14	5.183271	3.785537	1.397734	2.708338	0.269663	sm
15	5.183167	3.993087	1.190081	3.355307	0.229605	st
16	5.165159	3.464543	1.700615	2.037229	0.329247	sm
17	5.150733	2.818269	2.332463	1.20828	0.452841	m
18	5.121788	3.374803	1.746986	1.931787	0.341089	sm
19	5.06269	2.952966	2.109725	1.399692	0.41672	m
20	4.944227	3.310648	1.633579	2.026623	0.330401	sm
21	4.881902	3.43552	1.446382	2.37525	0.296274	sm
22	4.854176	2.546584	2.307591	1.103568	0.475383	m
23	4.738167	3.384844	1.353323	2.501134	0.285622	sm
24	4.730992	3.610128	1.120863	3.220846	0.236919	st
25	4.699862	2.358741	2.341121	1.007526	0.498126	m
26	4.696727	2.484985	2.211742	1.123542	0.470911	m
27	4.648584	2.884116	1.764468	1.634553	0.379571	m
28	4.642313	2.608927	2.033386	1.283046	0.438011	m
29	4.624318	3.154694	1.469624	2.1466	0.317803	sm
30	4.591675	2.485224	2.106451	1.179816	0.458754	m
31	4.536298	3.349722	1.186575	2.823018	0.261573	sm
32	4.532139	2.744025	1.788115	1.534591	0.394541	m
33	4.493448	2.846569	1.646879	1.728463	0.366507	sm
34	4.480478	3.181066	1.299412	2.448082	0.290016	sm
35	4.464239	2.462174	2.002065	1.229817	0.448467	m
36	4.44794	2.8869	1.561041	1.849343	0.350958	sm
37	4.408027	2.380071	2.027956	1.17363	0.46006	m

Chromosome number	Length each	Long arm	Short arm	Arm Ratio (L/S)	Cent. Index (S/(L+S))	Chromosome form
38	4.4047	3.069123	1.335577	2.297974	0.303216	sm
39	4.398596	2.845305	1.553291	1.831791	0.353133	sm
40	4.366992	2.304376	2.062616	1.11721	0.47232	m
41	4.360565	2.193641	2.166925	1.012329	0.496937	m
42	4.340465	3.357402	0.983062	3.415249	0.226488	st
43	4.301189	2.949489	1.3517	2.18206	0.314262	sm
44	4.261131	2.943733	1.317397	2.234506	0.309166	sm
45	4.234784	2.847409	1.387374	2.052373	0.327614	sm
46	4.197313	2.468205	1.729108	1.427444	0.411956	m
47	4.14596	2.439873	1.706086	1.430099	0.411506	m
48	4.136234	3.047852	1.088382	2.800352	0.263134	sm
49	4.07262	2.106115	1.966505	1.070993	0.48286	m
50	4.001428	2.036053	1.965375	1.035961	0.491168	m
51	3.992018	3.112741	0.879278	3.540111	0.220259	st
52	3.973599	2.64085	1.33275	1.981504	0.335401	sm
53	3.941663	2.933497	1.008166	2.909737	0.255772	sm
54	3.934908	2.365153	1.569756	1.506701	0.398931	m
55	3.915474	2.012021	1.903452	1.057038	0.486136	m
56	3.873676	2.370962	1.502714	1.577786	0.38793	m
57	3.854457	2.563633	1.290824	1.986044	0.334891	sm
58	3.850358	3.062533	0.787825	3.887325	0.204611	st
59	3.781247	2.692049	1.089198	2.471588	0.288053	sm
60	3.743094	2.252766	1.490328	1.511591	0.398154	m
61	3.679102	1.997974	1.681128	1.188472	0.45694	m
62	3.666277	1.9015	1.764777	1.077473	0.481354	m
63	3.608999	1.924108	1.684891	1.141978	0.466858	m
64	3.359084	1.700494	1.65859	1.025265	0.493763	m
65	3.262084	1.884994	1.37709	1.368824	0.422151	m
66	2.819974	1.487224	1.33275	1.115907	0.472611	m
Totals for set:	302.2207	189.1372	113.0835			
Average for set	4.579					

Appendix 3: Chromosome measurements (chromosome length, arm length, arm ratio and centromere position) of *Commelina diffusa* from Ginchi

Image magnification: 2200

Image resolution: 78.4 pixels per centimeter

Chromosome number	Length each	Long arm	Short arm	Arm Ratio (L/S)	Cent. Index (S/(L+S))	Chromosome form
1	7.816866	4.449783	3.367084	1.32155	0.430746	m
2	7.354533	5.159447	2.195087	2.35045	0.298467	sm
3	7.09962	5.188246	1.911375	2.71441	0.269222	sm
4	7.011956	3.98417	3.027787	1.31587	0.431804	m
5	6.845245	4.450552	2.394694	1.85851	0.349833	sm
6	6.783338	4.572836	2.210501	2.06869	0.325872	sm
7	6.596434	3.728901	2.867533	1.30039	0.43471	m
8	6.541437	4.776006	1.765428	2.7053	0.269884	sm
9	6.485326	4.788214	1.69711	2.82139	0.261685	sm
10	6.361478	3.787261	2.574217	1.47123	0.404657	m
11	6.251368	3.430194	2.821175	1.21587	0.451289	m
12	6.038683	3.988368	2.050317	1.94524	0.33953	sm
13	5.966339	4.427956	1.538384	2.87832	0.257844	sm
14	5.946804	3.980559	1.966247	2.02445	0.330639	sm
15	5.881509	3.149713	2.731799	1.15298	0.464472	m
16	5.773259	4.170174	1.603085	2.60134	0.277674	sm
17	5.732725	4.078657	1.654069	2.46583	0.288531	sm
18	5.694586	3.915011	1.779575	2.19997	0.312503	sm
19	5.670817	4.453056	1.21776	3.65676	0.214742	st
20	5.647288	4.03643	1.610857	2.50577	0.285244	sm
21	5.575229	3.621749	1.953479	1.854	0.350385	sm
22	5.561154	4.336571	1.224583	3.54126	0.220203	st
23	5.510451	4.432014	1.078437	4.10967	0.195707	st
24	5.509988	3.952963	1.557025	2.53879	0.282582	sm
25	5.45836	4.257131	1.201229	3.54398	0.220071	st
26	5.451846	4.69256	0.759285	6.18023	0.139271	st
27	5.420243	3.626633	1.79361	2.02197	0.330909	sm
28	5.398843	3.228691	2.170152	1.48777	0.401966	m
29	5.392164	3.038994	2.353169	1.29145	0.436405	m
30	5.31509	2.679566	2.635524	1.01671	0.495857	m
31	5.308546	4.315367	0.99318	4.345	0.187091	st
32	5.279856	3.801713	1.478143	2.57195	0.279959	sm
33	5.261636	2.836186	2.42545	1.16934	0.460969	m
34	5.242715	4.096859	1.145856	3.57537	0.218562	st
35	5.229873	3.628849	1.601025	2.26658	0.306131	sm
36	5.209988	3.73483	1.475158	2.53182	0.28314	sm
37	5.12288	2.566413	2.556467	1.00389	0.499029	m

Chromosome number	Length each	Long arm	Short arm	Arm Ratio (L/S)	Cent. Index (S/(L+S))	Chromosome form
38	5.110033	2.720592	2.38944	1.13859	0.467598	m
39	5.107175	3.508721	1.598454	2.19507	0.312982	sm
40	5.103431	2.776319	2.327112	1.19303	0.45599	m
41	5.076757	4.203183	0.873574	4.81148	0.172073	st
42	5.004744	2.617926	2.386819	1.09683	0.476911	m
43	4.987978	3.744493	1.243486	3.01129	0.249297	st
44	4.921818	2.688682	2.233136	1.20399	0.453722	m
45	4.873934	3.8858	0.988134	3.93246	0.202738	st
46	4.795971	2.51372	2.282251	1.10142	0.475868	m
47	4.791852	3.525792	1.26606	2.78485	0.264211	sm
48	4.756108	2.789385	1.966723	1.41829	0.413515	m
49	4.74082	2.450675	2.290144	1.0701	0.483069	m
50	4.697139	3.368154	1.328984	2.53438	0.282935	sm
51	4.688354	2.64564	2.042715	1.29516	0.4357	m
52	4.629819	3.023633	1.606187	1.88249	0.346922	sm
53	4.62857	3.331309	1.297261	2.56795	0.280273	sm
54	4.580349	3.526923	1.053426	3.34805	0.229988	st
55	4.545656	2.620361	1.925296	1.36102	0.423546	m
56	4.527285	2.558739	1.968547	1.29981	0.434818	m
57	4.50259	2.386	2.116591	1.12728	0.470083	m
58	4.437289	2.501054	1.936236	1.29171	0.436356	m
59	4.425088	2.421038	2.00405	1.20807	0.452884	m
60	4.341786	2.436726	1.90506	1.27908	0.438773	m
61	4.307519	2.451413	1.856106	1.32073	0.430899	m
62	4.253511	2.235935	2.017576	1.10823	0.474332	m
63	4.198898	3.372367	0.82653	4.08015	0.196845	st
64	4.050322	2.16542	1.884903	1.14882	0.465371	m
65	3.286648	2.373898	0.91275	2.60082	0.277715	sm
66	2.973389	1.97686	0.996529	1.98374	0.335149	sm
Totals for set	351.0933	230.1834	120.9099			
Average for set	5.319596					

Appendix 4: Chromosome measurements (chromosome length, arm length, arm ratio and centromere position) of *Commelina africana* from Sebeta

Image magnification: 2200

Image resolution: 78.4 pixels per centimeter

Chromosome number	Length each	Long arm	Short arm	Arm Ratio (L/S)	Cent. Index (S/(L+S))	Chromosome form
1	5.28762	3.715391	1.572229	2.363137	0.297341	sm
2	4.723674	3.987791	0.735883	5.419058	0.155786	st
3	4.451864	2.377199	2.074664	1.145824	0.466021	m
4	4.449179	2.799481	1.649698	1.696966	0.370787	m
5	4.383845	2.515686	1.868159	1.346613	0.426146	m
6	4.095369	3.342696	0.752673	4.441101	0.183786	st
7	4.009876	3.137256	0.87262	3.595214	0.217618	st
8	3.987608	2.369601	1.618007	1.464519	0.405759	m
9	3.943064	2.902369	1.040696	2.788874	0.263931	sm
10	3.779648	2.623621	1.156027	2.269516	0.305856	sm
11	3.776715	2.156025	1.62069	1.330313	0.429127	m
12	3.753604	2.451215	1.302389	1.88209	0.34697	sm
13	3.682839	2.905661	0.777179	3.73873	0.211027	st
14	3.522184	2.201856	1.320328	1.667658	0.374861	m
15	3.458233	1.759909	1.698324	1.036262	0.491096	m
16	3.297387	1.986081	1.311307	1.514582	0.39768	m
17	3.268558	1.875007	1.393551	1.345488	0.426351	m
18	3.236322	2.081119	1.155204	1.801517	0.35695	sm
19	3.233423	2.137562	1.095862	1.950576	0.338917	sm
20	3.204337	1.911152	1.293185	1.477865	0.403573	m
21	3.147463	1.770411	1.377052	1.285654	0.437512	m
22	3.060325	2.45971	0.600615	4.095321	0.196258	st
23	3.048591	1.572326	1.476265	1.06507	0.484245	m
24	3.028902	2.046975	0.981927	2.08465	0.324186	sm
25	2.907786	2.487525	0.420262	5.918989	0.14453	st
26	2.736564	1.491297	1.245267	1.197572	0.455048	m
27	2.699033	1.807505	0.891528	2.027423	0.330314	sm
28	2.689938	1.736998	0.952939	1.82278	0.354261	sm
29	2.011113	1.308237	0.702877	1.861259	0.349497	sm
30	2.00449	1.190913	0.813577	1.463798	0.405877	m
Totals for set:	104.8796	69.10858	35.77098			
Average for set	3.495985					

Appendix 5: Chromosome measurements (chromosome length, arm length, arm ratio and centromere position) of *Commelina diffusa* from Entoto mountain

Image magnification: 2200

Image resolution: 78.4 pixels per centimeter

Chromosome No.	Length each	Long arm	Short arm	Arm Ratio (L/S)	Cent. Index (S/(L+S))	Chromosome form
1	8.552728	5.274766	3.277964	1.609159	0.383265	m
2	8.422807	6.840684	1.582124	4.323734	0.187838	st
3	8.373693	4.742094	3.631597	1.305788	0.433691	m
4	7.775262	4.477966	3.297295	1.358073	0.424075	m
5	7.744903	4.332337	3.412566	1.269525	0.440621	m
6	7.67182	5.912537	1.759284	3.360764	0.229318	st
7	7.551227	4.650515	2.900712	1.603232	0.384138	m
8	7.423627	4.642126	2.781498	1.66893	0.374682	m
9	7.300771	5.725123	1.575651	3.633496	0.21582	st
10	7.268179	4.136536	3.131642	1.320884	0.43087	m
11	7.246254	5.123558	2.122695	2.413704	0.292937	sm
12	6.79531	4.258988	2.536324	1.679197	0.373246	m
13	6.709989	5.701825	1.008166	5.655643	0.150248	st
14	6.21672	4.801484	1.415237	3.392707	0.22765	st
15	6.057156	4.647849	1.409305	3.297972	0.232668	st
16	6.031363	4.516078	1.515282	2.980355	0.251234	sm
17	6.014374	3.515262	2.499113	1.406604	0.415523	m
18	5.94845	3.706301	2.242151	1.653011	0.37693	m
19	5.802209	3.029374	2.772836	1.092518	0.477893	m
20	5.705055	4.146416	1.55864	2.660279	0.273203	sm
21	5.632886	4.067059	1.565828	2.597386	0.27798	sm
22	5.388933	2.905881	2.483051	1.170286	0.460769	m
23	5.380869	3.165069	2.215799	1.42841	0.411792	m
24	5.246482	2.640276	2.606206	1.013073	0.496753	m
25	5.140992	2.642467	2.498526	1.05761	0.486001	m
26	4.997726	3.650066	1.34766	2.708448	0.269655	sm
27	4.889515	4.03718	0.852335	4.736614	0.174319	st
28	4.823204	2.821733	2.00147	1.40983	0.414967	m
29	4.592742	3.367926	1.224816	2.749741	0.266685	sm
30	4.487215	3.710572	0.776642	4.77771	0.173079	st
Totals for the set	191.1925	127.1901	64.00242			
Average for set	6.373082					

Appendix 6: Chromosome measurements (chromosome length, arm length, arm ratio and centromere position) of *Commelina diffusa* from Jimma

Image magnification: 2200

Image resolution: 78.4 pixels per centimeter

Chromosome number	Length each	Long arm	Short arm	Arm Ratio (L/S)	Cent. Index (S/(L+S))	Chromosome form
1	4.804788	2.829844	1.974944	1.432873	0.411037	m
2	4.768837	3.787471	0.981366	3.859388	0.205787	st
3	4.45922	2.797455	1.661765	1.683424	0.372658	m
4	4.403485	3.09795	1.305536	2.372934	0.296478	sm
5	4.16564	2.30236	1.86328	1.235649	0.447297	m
6	4.164756	2.892134	1.272621	2.272581	0.305569	sm
7	4.13725	3.140721	0.996529	3.151659	0.240868	st
8	4.114014	2.223875	1.890138	1.176567	0.459439	m
9	4.07338	3.175352	0.898027	3.535919	0.220463	st
10	4.04252	2.466456	1.576063	1.564947	0.389872	m
11	4.037343	3.298072	0.739271	4.461247	0.183108	st
12	3.962542	3.177365	0.785177	4.046684	0.19815	st
13	3.927951	3.193202	0.73475	4.345972	0.187057	st
14	3.763481	2.710056	1.053426	2.572611	0.279907	sm
15	3.720127	2.816551	0.903577	3.117112	0.242889	st
16	3.708144	2.984822	0.723322	4.126548	0.195063	st
17	3.704437	2.892598	0.811839	3.56302	0.219153	st
18	3.70239	2.201478	1.500912	1.46676	0.40539	m
19	3.426047	1.79327	1.632777	1.098294	0.476578	m
20	3.373676	2.405988	0.967687	2.486329	0.286835	sm
21	3.366662	1.783649	1.583012	1.126744	0.470202	m
22	3.358462	2.040158	1.318304	1.547562	0.392532	m
23	3.324125	2.227305	1.09682	2.030692	0.329958	sm
24	3.305687	2.608164	0.697523	3.739181	0.211007	st
25	3.292461	1.966139	1.326322	1.482399	0.402836	m
26	3.267515	2.327781	0.939734	2.477064	0.287599	sm
27	3.12111	2.122334	0.998776	2.124934	0.320007	sm
28	3.077926	2.382796	0.69513	3.427844	0.225844	st
29	2.97818	2.151649	0.82653	2.603231	0.277529	sm
30	2.83148	2.30834	0.52314	4.412467	0.184759	st
Totals for set:	112.3836	78.10533	34.2783			
Average per set	3.746121					

Appendix 7: Chromosome measurements (chromosome length, arm length, arm ratio and centromere position) of *Tradescantia zebrina*

Image magnification: 2200

Image resolution: 78.4 pixels per centimetre

Chromosome rank	Length each	Long arm	Short arm	Arm Ratio (L/S)	Cent. Index (S/(L+S))	Chromosome form
1	14.71803	7.573441	7.14459	1.060025	0.485431	m
2	13.85058	7.825727	6.024852	1.298908	0.434989	m
3	13.66855	7.037061	6.631492	1.061158	0.485164	m
4	11.94747	6.253637	5.693836	1.098317	0.476572	m
5	9.567697	8.727169	0.840523	10.38302	0.08785	t
6	9.238212	7.818309	1.419906	5.506215	0.153699	st
7	8.959857	7.167612	1.792244	3.999239	0.20003	st
8	8.733958	7.983502	0.750456	10.6382	0.085924	t
9	8.617331	7.956023	0.661302	12.03084	0.076741	t
10	8.258919	7.43239	0.82653	8.992277	0.100077	t
11	8.149191	7.257314	0.891881	8.137086	0.109444	t
12	7.8792	6.132052	1.747146	3.509753	0.221742	st
13	7.768785	7.093103	0.675682	10.4977	0.086974	t
14	7.701365	6.874836	0.82653	8.317705	0.107323	t
15	7.694432	7.100088	0.59434	11.94618	0.077243	t
16	7.493685	6.683285	0.8104	8.246894	0.108144	t
17	7.353806	6.594517	0.759285	8.685162	0.103251	t
18	7.347098	6.814874	0.53222	12.80461	0.07244	t
19	7.065405	5.649778	1.415625	3.991013	0.20036	st
20	6.363804	5.624532	0.739271	7.60821	0.116168	t
21	6.329081	5.057765	1.271314	3.978377	0.200869	st
22	6.202179	5.086837	1.115342	4.560786	0.179831	st
23	5.897014	5.380685	0.516329	10.42103	0.087558	t
24	5.508873	5.032841	0.476032	10.57248	0.086412	t
Totals for set:	162.1574	44.15713				
Average for set	8.596438					

Appendix 8: Chromosome measurements (chromosome length, arm length, arm ratio and centromere position) of *Commelina subulata*

Image magnification: 2200

Image resolution: 78.4 pixels per centimetre

Chromosome rank	Length each	Long arm	Short arm	Arm Ratio (L/S)	Cent. Index (S/(L+S))	Chromosome form
1	4.389088	2.561113	1.827974	1.401066	0.416482	m
2	4.103364	2.066557	2.036807	1.014606	0.496375	m
3	4.020322	2.343484	1.676837	1.397562	0.41709	m
4	3.899626	2.005821	1.893805	1.059148	0.485638	m
5	3.654891	2.027788	1.627103	1.246257	0.445185	m
6	3.57445	2.093338	1.481112	1.413355	0.414361	m
7	3.493551	2.348338	1.145214	2.050567	0.327808	sm
8	3.462134	2.257252	1.204881	1.873423	0.348017	sm
9	3.364172	1.978027	1.386144	1.427	0.412031	m
10	3.351091	2.142601	1.208491	1.772956	0.360626	sm
11	3.346791	2.593716	0.753075	3.444169	0.225014	st
12	3.292969	1.83768	1.455289	1.26276	0.441938	m
13	3.223312	1.893446	1.329865	1.423788	0.412577	m
14	3.19249	1.752611	1.439879	1.217193	0.451021	m
15	3.078034	1.682166	1.395868	1.205104	0.453493	m
16	2.976896	1.668723	1.308173	1.275614	0.439442	m
17	2.929659	1.696601	1.233058	1.375929	0.420888	m
18	2.866758	1.525283	1.341474	1.13702	0.467941	m
19	2.836518	1.955348	0.881171	2.219035	0.310652	sm
20	2.765186	2.177901	0.587285	3.708423	0.212385	st
21	2.736088	1.782254	0.953834	1.868517	0.348612	sm
22	2.731718	1.650329	1.081389	1.526119	0.395864	m
23	2.675613	1.587984	1.087628	1.460043	0.406497	m
24	2.51991	1.678396	0.841514	1.994496	0.333946	sm
25	2.518916	1.274472	1.244444	1.024129	0.49404	m
26	2.461121	1.758244	0.702877	2.501495	0.285592	sm
27	2.435543	1.695711	0.739832	2.29202	0.303765	sm
28	2.281995	1.362679	0.919316	1.482275	0.402856	m
29	2.175793	1.501963	0.67383	2.228995	0.309694	sm
30	2.166297	1.507471	0.658826	2.288116	0.304125	sm
Totals for set:	92.5243	56.4073	36.117			
Average per set	3.084143					

Appendix 9: Chromosome measurements (chromosome length, arm length, arm ratio and centromere position) of *T. fluminensis* (green)

Image magnification: 2200

Image resolution: 78.4 pixels per centimeter

Chromosome number	Length each	Long arm	Short arm	Arm Ratio (L/S)	Cent. Index (S/(L+S))	Chromosome form
1	6.865034	6.169903	0.69513	8.875904	0.101257	t
2	6.708962	5.841129	0.867833	6.730705	0.129354	st
3	6.527669	5.580867	0.9468	5.894454	0.145044	st
4	6.160684	5.580532	0.580153	9.619063	0.09417	t
5	5.779712	5.075057	0.704653	7.202209	0.121918	t
6	5.777652	4.973601	0.80405	6.185689	0.139165	st
7	5.728582	5.196362	0.53222	9.763557	0.092906	t
8	5.435053	4.132653	1.3024	3.173106	0.23963	st
9	5.236093	4.74287	0.493223	9.616076	0.094197	t
10	4.337795	3.968159	0.369636	10.73533	0.085213	t
11	3.974263	2.445823	1.52844	1.600209	0.384585	m
12	3.949568	2.394452	1.555115	1.539727	0.393743	m
13	3.883144	3.518044	0.3651	9.635835	0.094022	t
14	3.714519	3.182299	0.53222	5.979289	0.143281	st
15	3.694956	2.869432	0.825524	3.475893	0.223419	st
16	3.684873	3.245235	0.439639	7.381595	0.119309	t
17	3.576483	2.881354	0.69513	4.14506	0.194361	st
18	3.566416	2.684327	0.882089	3.043147	0.247332	st
19	3.531124	2.962576	0.568549	5.210768	0.161011	st
20	3.380071	2.835472	0.544599	5.206532	0.161121	st
21	3.349167	2.998025	0.351142	8.537928	0.104845	t
22	3.33313	2.963494	0.369636	8.017338	0.110897	t
23	3.298348	2.822315	0.476032	5.928832	0.144324	st
24	3.294239	1.977852	1.316387	1.502485	0.399603	m
25	3.272343	2.678004	0.59434	4.505846	0.181625	st
26	3.236755	2.206826	1.029929	2.142697	0.318198	sm
27	3.231057	2.884693	0.346364	8.32849	0.107198	t
28	3.204366	1.724059	1.480307	1.164663	0.461966	m
29	3.13229	1.986419	1.145871	1.733545	0.365825	sm
30	3.131733	2.744486	0.387247	7.08717	0.123653	t
31	3.084585	2.790232	0.294353	9.4792	0.095427	t
32	3.064607	2.484454	0.580153	4.282409	0.189308	st
33	3.046452	2.388258	0.658193	3.628506	0.216052	st
34	3.039351	2.53745	0.501901	5.055682	0.165134	st
35	3.037529	2.342399	0.69513	3.36973	0.228847	st
36	3.028564	2.734212	0.294353	9.288883	0.097192	t
37	3.025218	1.835124	1.190095	1.541998	0.393391	m

Chromosome number	Length each	Long arm	Short arm	Arm Ratio (L/S)	Cent. Index (S/(L+S))	Chromosome form
38	2.998839	1.94472	1.054119	1.844876	0.351509	sm
39	2.995368	2.426819	0.568549	4.268444	0.189809	st
40	2.947666	2.653312	0.294353	9.014046	0.09986	t
41	2.927451	1.530909	1.396542	1.096214	0.47705	m
42	2.913253	2.618899	0.294353	8.897135	0.101039	t
43	2.890438	1.988708	0.901731	2.205435	0.31197	sm
44	2.881826	2.465548	0.416278	5.92284	0.144449	t
45	2.861834	1.938195	0.923638	2.098436	0.322744	sm
46	2.847258	2.174046	0.673211	3.229368	0.236442	st
47	2.818449	1.540443	1.278006	1.205348	0.453443	m
48	2.763484	2.474847	0.288637	8.574256	0.104447	t
49	2.735294	2.424422	0.310872	7.798792	0.113652	t
50	2.700532	1.847597	0.852935	2.166164	0.31584	sm
51	2.682941	2.438025	0.244917	9.954512	0.091287	t
52	2.638277	1.656912	0.981366	1.688374	0.371972	m
53	2.636835	2.342482	0.294353	7.958068	0.111631	t
54	2.605592	2.374682	0.23091	10.28404	0.088621	t
55	2.546988	2.252635	0.294353	7.652833	0.115569	t
56	2.499004	2.104671	0.394333	5.337294	0.157796	st
57	2.473246	2.007833	0.465413	4.314088	0.188179	st
58	2.42257	2.214431	0.208139	10.63919	0.085917	t
59	2.292006	1.875728	0.416278	4.505949	0.181622	st
60	2.248932	1.809292	0.439639	4.115408	0.195488	st
Totals for the set	211.6505	171.4836	40.16686			
Average for set	3.527508					

Declaration

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

Name:-_____

Signature:-_____

Date:-_____

This thesis has been submitted to examination with the approval as University advisors

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Kifle Dagne (Dr.)

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Ensermu Kelbessa (Prof.)