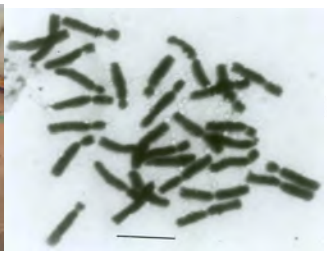
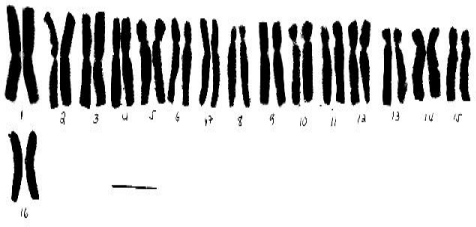
SOMATIC CHROMOSOME STUDY OF SOME SELECTED AFROALPINE PLANTS FROM BALE MOUNTAINS, ETHIOPIA.

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BY

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

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ABSTRACT

Afroalpine plants of the afroalpine environment, the scattered high mountains of Ethiopia and Tropical East Africa, have not been widely studied. Ten species of afroalpine plants were sampled randomly from Bale Mountains and their mitotic chromosomes have been studied or determined using an air-dry method. For chromosome preparation, root tips were pretreated with ice-cold water (24hr) or colchicine (3–4hr), fixed in 3:1 (ethanol:glacial acetic acid) and air-dry slide preparation was made following cellulose-pectinase maceration (37^{o}C,1hr) and stained in Giemsa. For the karyotypic analysis, photographs were taken from the best metaphase plates of each specimen. Ideogram was taken and chromosomes types were determined according to Levan *et al*., criterion. The result showed that, the chromosome numbers of 2n = 20 for *Centella asiatica* L.; 2n = 72 for *Cotula cryptocephala*; 2n = 40 for *Senecio nanus*; 2n = 56 for *Crassula alsinoides*; 2n = 28 for *Geranium arabicum*; 2n = 28 for *Lobelia rhychopetalum*; 2n = 12 for *Plantago major*; 2n = 18 for *Rumex nepalensis*; 2n = 32 for *Ranunculus oreophytus* and 2n = 32 for *Ranunculus multifidus*. Karyotypes have been constructed for *Senecio nanus*, *Plantago major* and *Ranunculus oreophytus* species. The first two species have symmetrical karyotype consisting metacentric chromosomes, whereas the latter species showed asymmetrical karyotype with metacentric, sub-metacentric and sub-telocentric chromosomes. The chromosomes length of *Senecio nanus*, *Plantago major* and *Ranunculus oreophytus* range between 2.28 and 11.35µm. Since detailed karyotypic analysis could not be given to the most species under the present study, it is better to carry out further cytological study in the future to get more reliable chromosome data for better understanding about their taxonomic and evolutionary relationships.

Key words: Afroalpine environment, Afroalpine plants, Bale Mountains, Chromosome numbers, Chromosomes length, Karyotype, Ideogram.

opportunities for the study of speciation in connection with geographic isolation and adaptation to extreme environmental condition (Hedberg, 1970).

There is a steep climate gradient along the mountain slopes, resulting in distinct vegetation belts that were experienced altitudinal shifts in response to the glacial cycles of the Pleistocene. Therefore, along the slopes of these 'sky islands', the vegetation is divided into three altitudinal zones (Hedberg, 1951): the afro-montane zone, the sub-alpine ericaceous zone and the afro-alpine zone. Similarly Mohammed and Bonnefille (1998) have shown the presence of the above three vegetation belts namely, the montane forest zone, the ericaceous zone and the afroalpine zone.

The afroalpine flora is occurring in isolated enclaves on the upper parts of the high mountains of Ethiopia and Tropical East Africa (Kenya, Tanzania and Uganda; Hedberg, 1975). Hedberg (1986) also defined an afroalpine plant as a species, which has been found in the Afroalpine belt on one or more East African mountains.

The afroalpine flora which is poor in species and peculiarly adapted to the extreme diurnal climate. Phytogeography of this afroalpine flora is complex derivation because no less than of 80 percent of its taxa are endemic to the high mountains of Tropical East Africa and Ethiopia. Moreover, most of afroalpine pants have closest relatives, not on the lower parts of the mountains, nor in their surroundings, but in distance parts of the world, such as in the temperate parts of the northern or southern hemisphere, in south Africa, in the Mediterranean area, or along the Himalaya (Hedberg,1965).

The biogeographical history of alpine plants, however, is often poorly known. Many of these plants are suggested to have originated from migrants pre-adapted to high-altitude climates (Magnus, *et al*., 2008). According to Billings (1974) it is believed that, most alpine plants have been formed by evolution and migration during the late Tertiary and Pleistocene on new mountains which provided open environments and selected more members of other floras which where adapted or could adapt to low-temperature stress (Koch *et al*., 2001, 2006).

The vicarious species of the afroalpine flora have evidently arisen under influence of geographical isolation. These mountains have been isolated from each other high mountains areas for a very long time, and thus dispersal of plants between them must presumably have occurred mainly by long distance, possibly facilitated by cyclones (Hedberg, 1969).

The differentiation of the vicarious species of afroalpine flora is assumed to have occurred through natural selection in connection with genetic drift, acting upon geographically isolated and originally very small random samples of the gene pools concerned. The amount of differentiation between different mountain populations differs considerably between different groups. The rate of evolutionary change seems to differ considerably between different genera and families (Hedberg, 1969).

The characteristics (physical and biological) which alpine plants possess have resulted from the interaction between alpine environment and available genetic system. In alpine ecosystem, the right combination of morphological, physiological and reproductive characteristics at the time of environmental stress tend the alpine plants to adapt to peculiar tropical-alpine climate, which includes frequent night frosts, rapid heating by intensive radiation and certain amount of frost heaving (Hedberg, 1957).

The afroalpine flora consists of a wide variety of plants. However, the most common plants are herbs: perennial and annual; prostate shrubs; caulescent woody rosette plants and lichens; bryophytes and macro- fungi (Billings, 1974). There are about five distinct life forms adapted to tropical alpine condition, viz., giant rosette plants, tussock grasses, a caulescent rosette plants, cushion plants and sclerophyllous shrubs (Hedberg, 1964).

The karyological knowledge of the afro-alpine plants is essential to clarify the patterns of plant evolution in afro-alpine environments and also useful in tracing taxonomic and evolutionary relationships of afro-alpine plants. Despite the existence of earlier summaries of cytogenetic work, the current stage of karyological knowledge of the afro-alpine plants is far from satisfactory. Thus, chromosome counts on afro-alpine plants are

few and are usually based on very limited numbers of observations. Typically chromosome counts have been established from few populations (Hedberg and Hedberg, 1977)

Ethiopia has the largest extent of Afroalpine habitat in Africa (Yalden, 1983). The Ethiopian mountains are part of quite extensive highlands; the southern part of the region consists mainly of isolated peaks emerging from the lowlands and the elevated shoulders of the Great Rift Valley. Ethiopian afroalpine flora is poor in species but comprises species from different phytogeographic areas, such as Europe, Himalaya, Mediterran, etc. Although the first afroalpine known to science came from Ethiopia (Richard, 1986), the afroalpine flora of Ethiopia has not yet been satisfactory studied because of large-scale destruction of natural vegetation on most mountains. This has made delimitation of vegetation belts a difficult task (Hurni, 1982).

Cytologically, only a very few comprehensive studies have been carried out on the cytology of afroalpine plants of Ethiopia by Hedberg and Hedberg (1977). Therefore, in this study some chromosome data were generated on some selected Afroalpine plants from Bale Mountains that could contribute towards resolving the problems of classification and establishing the relationships among Afroalpine plants.

1.2. GENERAL DESCRIPTIONS OF PLANT SPECIES UNDER STUDY

Ten species of afroalipine plants from Bale Mountains, which belong to 8 families, were used in the present study of somatic chromosomes. Their general descriptions are given as follows.

1.2.1. *Centella asiatica* (L.) Urban. (1879), Apiaceae

The plant is a perennial herb. Stems are slender, long, creeping and rooting at the nodes. Leaves are simple, orbicular rein form, entire, crenate or lobulate. Flowers are 3-4 flowered umbels arising in auxiliary (Dawit Abebe *et al*., 2003) (Fig.1a). It is a weed growing in wet place (damp grassland or swampy areas) of tropical and subtropical regions at an altitudes ranging between 1000-3200masl. In Ethiopia, this species is found

in Tigray, Gondor, Gojam, Shewa, Arsi, Welega, Illubabor, Gamo Gofa, Sidamo, Hararge and Bale regions (Hedberg *et al*., 2003).

As long as our knowledge is concerned, no cytological studies have been found on the *Centella asiatica* so far. However, cytological study made by Bennett and Smith (1991) shows that some species of Apiaceae have basic chromosome numbers of 6, 8, 9, 10, 12, and 18 with ploidy level of 2x.

Chemicals in *Centella asiatica* have been proven to treat leprosy, syphilis, and skin diseases (Jansen, 1981) and as a remedy for headache (Tyler *et al*, 1988). It has also been used for wound healing, better circulation, memory enhancement, cancer, vitality, general tonic, respiratory ailments, detoxifying the body, treatment of skin disorders (such as psoriasis and eczema), revitalizing connective tissue, burn and scar treatment, clearing up skin infections, slimming and edema, arthritis, rheumatism, treatment of liver and kidneys, periodontal disease, strengthening of veins (varicose veins), blood purifier, high blood pressure, sedative, anti-stress, anti-anxiety, an aphrodisiac, immune booster, anabolic and adaptogen etc (Oliver- Bever, 1980).

1.2.2. *Cotula cryptocephala Sch*. *Bip*. ex *Rich* (1848), Asteraceae

The plant is an erect, perennial herb reaching 5 to 10 cm high (Fig. 1b). Leaves are opposite, bipinnatifid, oblong or ovate in outline, hirsute, 3-8.5 cm long including the sheathing, connate petiole. Central florets are bisexual and yellow, corolla are tubular and glandular. Their stem is simple or branched. It is widely grows on moist ground in short grass, along paths, roadsides margins and river banks at 2800-3750masl. In Ethiopia, it is grown in Gondor, Shewa and Bale regions; it is also found in Uganda and Kenya (Hedberg *et al*., 2004).

The earliest chromosome count in the literature for *Cotula cryptocephala* is that of Hedberg and Hedberg (1977). This paper representing 2n = 80 with basic chromosome number of x = 8. Genus *Cotula* is known to have basic chromosome numbers of x = 8 and 9 with ploidy of 4x, 5x, 6x, and 10x (Hedberg and Hedberg, 1977).

There are some reports on the role of *Cotula cryptocephala* in medication. Dawit Abebe *et al*., (2003) reported that it is used to treat tropical ulcer, cardiac disorder, typhoid fever, rheumatism, asthma, swelling, oxytic and also used to regulate menstruation and as sedative to the uterus (Kotb,1985). Insect anti-feedent and antibacterial activities were also reported by Harborne and Baxter (1993).

1.2.3. *Senecio nanus Sch. Bip*. ex. *A. Rich*. (1848), Asteraceae

It is a perennial herb of about 5-10(-28) cm high, with thick rootstock, rhizome mauve when slashed (Fig.1c). Leaves are simple, radial, narrowly oblong to slightly oblaneolate and fresh. Margins are either entire or indistinctly denticulate, incurred and apex is obtuse. Peduncle is 10cm long and arising from leaf rosette or from auxiliary branches. Ray florets are yellow. Its stem is short with numerous crowded leaves, rarely elongated and bearing dispersed leaves. This species, *Senecio nanus*, is very common on moist high altitude grassland, especially in depression of flat, rocky place; 3250-4100masl. In Ethiopia, this species is found in Gondor, Gojam, and Bale regions. No known elsewhere (Hedberg *et al*., 2004).

Chromosome count made by Hedberg and Hedberg (1977) indicated that *Senecio nanus* is a tetraploid plant with 2n = 40 chromosomes. According to the work of Bennett and Smith (1991), genus *Senecio* is also characterized by having basic chromosome numbers x = 4, 6 and 8 with ploidy of 8x and 10x.

Although Asteraceae is well known in its medicinal values, *Senecio nanus* has no any records.

1.2.4. *Crassula alsinoides* (Hook f.) Engl. (1892), Crassulaceae

Crassula alsinoides is a creeping perennial herb that is rooting from the lower nodes. Leaves are opposite; often journal bases into sheath; ± ovate with ill-defined platelets joined at base into short sheath; tip usually acute, apiculate; margin ± entire, often hyalim and/or with sub marginal dark dots on underside (Fig.1d). Flowers are usually solitary in leaf axils. It grows and lives at the altitude ranging between 1500-3100masl and specially

or usually in upland forest with junipers and/or *Hagenia*, sometimes on boulders or fallen logs and mostly in shade. In Ethiopia, this species is found in Shewa, Arsi, Kefa, Sidamo, Harerge and Bale regions (Hedberg and Edwards, 1989).

As far as our knowledge is concerned, there is no report on the cytology, as well as on the economic importance of *Crassula alsinoides*. But the genus *Crassula* is characterized by the basic chromosome numbers of x = 7, 8 (Hedberg and Hedberg 1977; Randle *et al.*, 2005). As far as ploidy level is concerned, Hedberg and Hedberg (1977) reported that 4x, 5x, 6x, 8x, and 12x could be the possible ploidy levels of this genus.

1.2.5. *Geranium arabicum* Forssk. (1775), Geraniaceae

Geranium arabicum is a perennial herb, sometimes small. Most of its parts are stiffly pubescent or pilose. Their stems are often stoloniferous, but sometimes scadent, at higher altitude forming dense tuffs. Leaves are opposite, sometimes crowded into basal rosette, stipules avate-lanceolate. The inflorescence (1-) 2-flowered with either white or pale pink color. It is among a very widely spread plants and variable species (Fig. 1e). Three sub-species have been recognized. It is believed that this plant occurs at the altitudes between 1300-3650masl in herb layer of forests, damp grassland, besides streams and afroalpine grassland. In Ethiopia this species is distributed in Tigray, Gonder, Gojam, Shewa, Arsi, Illubabor, Kefa, Sidamo, Bale and Harerge (Edwards *et al*., 2000).

Geranium arabicum is a diploid species (2x) characterizing by a chromosome number 2n = 28 with a basic chromosome number of x =14 (Hedberg and Hedberg, 1977). Hedberg and Hedberg (1977) have also proposed that x = 14 as a basic chromosome number and 2x and 4x as a possible ploidy levels of the family Geraniaceae.

To our knowledge, there is no literature on the economic significances of *Geranium arabicum* yet. However, some species are very widely grown as ornamentals, others are important sources of essential oils and still many are used locally as medicinal and dye plants.

1.2.6. *Lobelia rhynchopetalum* (Hemsl) (1877), Lobeliaceae

It is an erect, non-branching shrub up to 7m having stem crowded with large rosette leaves (Dawit Abebe *et al*., 2003). The leaves are alternatively arranged. Its florescence reaches up to several meters long dense and cylindrical (Fig.1f). This species is known to grow on upland, rocky mountainsides and often the only tall woody plant at high altitude; 3000-4350masl. In Ethiopia, this species is found in Gonder, Gojam, Shewa, Arsi, Harerge and Bale regions (Hedberg and Edwards, 1989).

Chromosome reports for some species of *Lobelia* were made by Bennett and Smith (1991) which showed that the basic chromosome numbers x = 6, 8, 10, 17 and ploidy levels of 2x, 3x, 4x of some species of *Lobelia*. Another study made by Hedberg and Hedberg (1977) indicates that some species of *Lobelia* were found to be diploid and tetraploid with basic chromosomes of x = 6, 7 and 13. The plant, *Lobelia rhynchopetalum*, is reported to have a chromosome number 2n = 28 and a tetraploid (4x) with a basic number of x = 7 (Hedberg and Hedberg, 1977).

Extract of *Lobelia rhynchopetalum* contains several alkaloids. It has been reported that various forms of preparations of the plant is employed in the treatment of gonorrhea, rabies, measles, scabies, chest pain, epilepsy, hemorrhoids and leshimaniasis (Dawit Abebe *et al*., 2003).

1.2.7. *Plantago major* L. (1753), Plantaginanceae

Plantago major is a plant of the family Plantaginaceae (Fig. 1g). This plant is a perennial herb with several adventitious roots from a short rhizome. Their leaves grow in basal rosettes, long petiolate to almost sub-sessile and leaf blade is elliptic or slightly ovate, with 5-7 parallel main veins, margin entire or remotely dentate or with remote small lobes. The flowers are small and greenish brown and usually bisexual. Spikes are linear-cylindrical; bract ovate and concave in shape. Corolla lobes are triangular and yellow-white in their shape and color, respectively. *Plantago major* is usually grown on moist roadsides and wet ground occurring at 1300-2400masl. In Ethiopia, this species is found

in Gonder, Shewa, Harerge and Bale regions. Native in Europe and central Asia, but now naturalized in most parts of the world (Hedberg *et al*., 2006).

We could not find any previous report on chromosome number of this species. However, in the literatures, it is indicated that most species of genus *Plantago* have the basic chromosome number of x = 6 and some like *Plantago insularis* have the basic chromosome number of x = 4 (Stebbins, 1971). Thus, no report is available on the ploidy, as well as chromosome number of *Plantago major*.

Although Plantaginanceae are well known in their economic importance (as food plants, herbal remedies, astringent, anti-toxic, antimicrobial, anti-inflammatory and medicinal plants (Dagar *et al*., 2006), we could not get a piece of information on the significances of *Plantago major*.

1.2.8. *Rumex napalensis* Spreng (1825), Polygonaceae

This plant is a stout, erect and perennial herb up to 2m tall having green or pale brownish stems. Leaves are very long up to 20 cm, basal in shape, with margin flat or wavy (Fig.1h). The inflorescences are branched and terminal panicle with clusters of flowers hanging dawn. Flowers are usually unisexual. *Rumex napalensis* is usually grown as a weed in disturbed habitats and afroalpine moorland at 1200-3900masl. It is a widely distributed most parts of Ethiopia, such as Tigray, Gonder, Gojam, Welo, Shewa, Arsi, Welega, Illubabor, Kefa, Gamo Gofa, Bale and Harerge regions. Out side Ethiopia, it is also found thought out Africa, Madagascar, South and East Asia (Edwards *et al*., 2000).

No one has yet reported on the ploidy, as well as chromosome number of *Rumex napalensis*. Hedberg and Hedberg (1977) noted that this family, Polygonaceae is characterized by the basic number x = 6, 7, 8, 9, 13 and 17 with a pliody of 4x. While the report made by Bennett and Smith (1991) points that some species of Polygonaceae are represented by basic chromosome numbers and ploidy levels x = 7, 10, 11 and 2x, 4x, respectively.

Although genus *Rumex* is well known for its economic importance, no information is available on the significances of *Rumex napalensis*.

1.2.9. *Ranunculus multifidus* Forssk (1775), Ranuncululaceae

It is a Perennial erect or rarely prostrate herb up to 1m long and rooting at some of the nodes. It is a spreading weed with leafy runner (Dawit Abebe *et al*., 2003). Leaves are borne near the base at the ground. They are basal and cauline; variable in size and shape; bi or tri-pinnatisect. Stems are pilose with adpressed hairs pointing upwards. Flowers are solitary and auxiliary or in leafy terminal inflorescences (Edwards *et al.*, 2000). They are brightly yellow in color and cup-shaped (Fig.1i).The plant is a common weed in the cultivated areas (Dawit Abebe *et al*., 2003) and also occur in moist and open grassy places, near rivers, streams and lakes, on wet scopes in open montane forest at 1200-3800masl. In Ethiopia, this species is found in Tigray, Gojam, Welo, Shewa, Arsi, Welega, Illubabor, Kefa, Sidamo, Harerge and Bale regions (Edwards *et al.*, 2000).

Thulin (1970) reported that, the chromosome numbers of *Ranunculus multifidus* is 2n = 32 which is a tetraploid plant with a basic number of x = 8. As Hedberg and Hedberg (1977) indicated, the possible basic chromosome numbers of this family, Ranunculaceae are x = 7, 8 and 9 and their ploidy levels are shown to be 4x, 8x, and 10x. While Bennett and Smith (1991) reported that x = 2, 8, and 4x could be among the possible basic chromosome numbers and ploidy of some species of Ranunculaceae, respectively.

It has so many medicinal significances. As indicated by Dawit Abebe *et al*. (2003), it has been extensively used to treat toothache, headache, hemorrhoids, eczema, gout, rheumatic, breast cancer, crysiples, prutitis, sciatica, arthritis and rhinitis. There is also a report that it is used to treat lung TB, swelling, leishmania and tropical cancer (Dawit Abebe *et al*., 2003). It is also applied as antifungal (Harbore and Baxter, 1993) and as antiumour agent (Dawit Abebe *et al*., 2003).

1.2.10. *Ranunculus oreophytus* Del. (1843), Ranuncululaceae

Ranunculus oreophytus consists of perennial herbs with a large base and well developed root system. Usually both the leaves and flowers are produced from the base, but sometimes with stems exceeding the leaves. Their leaves are about 5-12 with dilated bases and forming the rosette spreads over the ground, imparipinnate, sessile or shortly petiolate, dentate or incised margins and more or less elliptical in shape (Fig.1j). They occur in moist peaty places, streambeds, gravel and grassy meadows as well as afroalpine regions in association with *Alchemilla* and other herbs. They typically grow at altitudes ranging from 2500-4000masl. In Ethiopia, this species is found in Gondor, Welo, Gojam, Shewa, Arsi, Sidamo, Harerge and Bale regions (Edwards *et al*., 2000).

From the work of Hedberg and Hedberg (1977), it is well known that *Ranunculus oreophytus* is a tetraploid plant having a chromosome number of 2n = 32 with basic number x = 8.

To our knowledge, no report so far has been made on ethno-medical use of this particular plant, *Ranunculus oreophytus*.

1.3. CYTOGENETICS AND ITS IMPORTANCES IN SYSTEMATICS AND EVOLUTIONARY STUDIES.

For more many years, chromosome cytology has been an important element in evaluating relationships and deducing phylogenetic sequences in plants and animals, but the use of such data is not simple (Raven, 1975). Chromosome data are relevant to plant systematics and evolution ranging from simply the number of chromosomes to details of molecular cytogenetics that are at the frontiers of current research (Stace, 2000).

Chromosomes are useful to address a variety of taxonomic and evolutionary questions (Stuess, 1990), because they occur in sets of defined number and be counted, have defined shapes, show land-marks for individual identification and their content of DNA can be measured (Greilhuber and Ehrendorfer, 1988).

Chromosomes become more visible microscopically during mitosis when they shorten and thicken during metaphase as a result of increased coiling of the DNA and its associated histone. This is the primary stage when cytogenetic analysis is performed (Griffiths *et al*., 2000).

The study of chromosomes has become much easier in recent years because of technical improvements. Furthermore, new staining procedures have been developed that differentially color different parts of the chromosome, so that each chromosome has a characteristic pattern. Using these procedures, cytologist can easily recognize each chromosome of an organism (Crow, 1976).

1.3.1. Chromosome Number

The realization that most species possess a constant chromosome number came well over a century ago. Strasburger in 1882 was the first person to report a somatic chromosome number of 24 in four species of *Lilium*. Today, chromosome number reporting is still continues. However, a chromosome count exists for only very few plant species in each representative group. For example, to date chromosome numbers have been reported for only about 25% of angiosperms (Bennett, 1998; Stace, 2000). Moreover, this coverage is very patchy, unrepresentative and the information is incomplete and incorrect for many species, because many counts were made for only one individual or population and many species were misnamed (Solits *et al*., 2003). Stace (2000) has also reached the same conclusion that, in general, many anomalous counts in the literature are the result of misidentifications, abnormal cells and tissues.

In some plants, counting of chromosomes is very difficult and inaccurate because of their small size (Poma *et al*., 1998) and the accidental presence of B-chromosomes which occur in variable numbers (Stebbins, 1971).

The number of chromosomes in a species normally remains constant through successive generations and this results in constancy of characters (Sinha and Sinha, 1976). There is, however, a great diversity in the number of chromosomes among different species. In

plants, for example, the number of somatic chromosome varies between 2n = 4 (in *Haplopappus gracilis*, Compositae) and 2n = 1440 (in some Ptredophytes) (Stace, 2000; Gupta, 2004). Related species may or may not have the same chromosome numbers (Sharma, 1991). The more closely related species are the more likely to have the same chromosome number and the more distantly related species are the more likely to have different chromosome numbers (Stace, 2000).

1.3.1.1. Ploidy and Basic Chromosome Number

Griffiths *et al*. (2000) defined ploidy as the number of chromosome sets in a cell. An increase and decrease in ploidy level seem to occur in organism. For example, fertilization between unreduced and with normal haploid reduced gametes leads to increase in ploidy level (Husband, 2004). According to Jackson (1971) new ploidy level may be produced as a result of eupolidy or polyploidy.

Ploidy determinations have traditionally been done only by chromosome counts, but this is laborious. More recently, flow cytometry which is rapid, easy and convenient method has become a commonly used technique for determining the DNA content of plant nuclei and deducing ploidy level (Brummer *et al*., 1999).

Basic chromosome number is the number of chromosomes in a set (Snustad *et al*., 1997). Since each species has its own specific base number (s), it is one of a widely used character in biosystematic studies and there have been a vast amount of phylogenetic speculation using this value as a guide (Jones, 1970). Chromosome base-numbers are, therefore, frequently of great evolutionary significance and taxonomic value (Raven, 1975).

A wide range of base-numbers may occur in some groups of plants. In the *poaceae*, for example, the subfamilies are characterized by different base-numbers. The three most common routes are probably loss of small chromosomes following unequal translocations, aneuploidy at polyploidy level and dibasic polyploidy. Centric fusions also give rise to new and different base-numbers (Hollings and Stace, 1974).

1.3.2. Chromosome Morphology

Chromosome morphology is studied on somatic chromosomes (Jackson, 1971), because at somatic metaphase the chromosomes become contracted to the maximum or nearly so and they are most easily studied (Snustad *et al*., 1997; Sharma, 1991).

Such studies on chromosomes have been shown that, chromosomes vary greatly in their gross morphology. The most obvious features defining chromosome morphological variations are total length and relative length of their arms, number and position of constrictions and other gross morphological features (Stace, 2000).

Many chromosomes have a distinctive morphology which makes it possible their individual recognition and identification of the karyotypes to which they belong (Lewis and John, 1963). The study of chromosome morphology is, therefore, a powerful tool in the resolution of taxonomic problems and in the tracing of evolutionary relationship (Stace, 1980; Smith, 1991).

1.3.2.1. Centromere

The centromere is one of the principal landmarks which may be seen on metaphase chromosomes to which the spindle fibers are attached (Stebbins, 1971). It is under-condensed region within a chromosome and is responsible for accurate segregation of the replicated chromosomes during mitosis and meiosis because it is associated with spindle fiber (McClean, 1997).

The relative position of the centromere may vary from chromosomes to chromosomes, but its position is constant for a given homologous pair, which serves as a basis for chromosome classification.

According to the position of the centromere, chromosomes are classified into the following types:-

1) Metacentric: - Chromosomes having median centromere, i.e. located at or near the middle of the chromosome (Stebbins, 1971; John, 1976).

2) Sub-metacentric: - Chromosomes having sub-terminal centromere i.e. located near one end of the chromosome (Stebbins, 1971; Singn, 2003).
3) Acrocentric: - Chromosomes having the centromere located near one end of the chromosomes whereby the chromosome possesses one long and one short arms (Stebbins, 1971; John, 1976).
4) Telocentric: - Chromosomes having terminal centromere whereby chromosomes have a single arm (Stebbins, 1971; John, 1976).

Longley (1941) classified chromosomes as terminal, sub-terminal, sub-median and median corresponding telomeric, acrocentric, sub-metacentric and metacentric position of centromere, respectively.

Making Longley's (1941) classification as a basis, Levan *et al*., (1964) have also developed a numerical system to classify chromosomes as Median point (M), median region (m), sub-median region (sm), sub-terminal region (st), terminal region (t), and terminal point (T), which correspond to the long : short arm ratios of 1.00, 1.00-1.69, 1.70-2.99, 3.00-6.69, 7.00- ∞ and ∞, respectively.

1.3.2.2. Telomere

Telomeres are the region of DNA at the end of the linear eukaryotic chromosome that are required for complete replication and stability of the chromosomes. They serve as a kind of cap that prevent the ends of chromosomes from attaching to the ends of other chromosomes. Scientists suspect that telomeres may influence the activity of nearby genes and also play a role in determining the life span of a cell (McClean, 1997).

1.3.2.3. Secondary Constriction and Satellite

Secondary constriction is also known as Nucleolar Organizing Regions, (NORs) (Stace, 2000). It is a constricted region located between the centromere and the end of the chromosome arm on one or few pairs of chromosomes (Weaver and Hendrick, 1992). Because it occupies a constant location, it serves as a morphological marker, which gives the chromosome its distinct morphology (John, 1976).

The part of the chromosome arm distal to the secondary constriction is known as the Satellite (Stebbins, 1971). As stated by Stace (2000), satellite is formed by secondary constriction on the nucleoli organizer chromosomes. It is one of the most important chromosomal land-markers that usually appear as a single small spherical body or a pair of such bodies attached to the remainder of the chromosome by a slender thread (Stebbins, 1971).

Species may differ in the number, size and position of satellites (Weaver and Hendrick, 1992). Differences in the number and position of satellites reflect differences in the location and size of nucleolar organizer regions (Stebbins, 1971).

1.3.3. Chromosome Size

As indicated by Crow (1976), chromosomes may vary in size between species of the same genus, between genera and between families (Jones, 1970). Stebbins (1971) has also noted that chromosome size may also vary to small extent in different tissues and organs of individual within a single organism. Size differences might be due to differential penetration of fixing and hydrolyzing chemicals or action of colchicines used in chromosome preparation (Jackson, 1971) or length wise replication of chromosome segment (Jones and Rees, 1982).

In general, plants have relatively larger chromosomes than animals. Among plants, chromosomes of monocots are larger than most of dicots and other plants (Sinha and Sinha, 1976). The karyotypes of most species of plants also consist of chromosomes, which are comparable to each other in size. In others, a graded series of chromosomal size exists as in many animals (Stebbins, 1971).

Lima De-Faria (1980) divided chromosomes of eukaryotic organisms into four grades: (I) length less than 1μm; (II) length between 1.0 and 4.0μm; (III) length between 4.0 and 12.0μm; and (IV) length more than 12.0μm. Most plants have chromosomes in the 3rd grade.

According to Jones (1970), the length of a chromosome is constant and chromosomes may arbitrary be classified as long (>10.0 µm), median (4.0-8.0 µm) or Short (< 2.0 µm). On the basis of their total length, chromosomes are also divided into four categories, small, medium-small, medium-large and large when their total lengths lie < 2.0 µm, 2.0 - 5.0 µm, 5.0 - 9.0 µm and >9.0 µm respectively (Stebbins, 1938).

The chromosomes are defined as being small, medium or large according to their normalize length. Large chromosomes have a value of 100 units or more (i.e. 10% or more of the total haploid complement); small chromosomes have a value of 80 or fewer units (8% or less of the total haploid complement); chromosomes whose length fall between 80 – 100 units are considered to be intermediate in size (Bogart, 1974).

1.3.4. Karyotype

A karyotype is a standardized arrangement of all the chromosomes of a cell (Lisa *et al*., 2005) or the phenotypic appearance of the somatic chromosomes in contrast to their genetic content (Stace, 2000), and its diagrammatic representation is called Karyogram or Ideogram (Grellhuber and Ehrendorfer, 1988).

Usually somatic karyotypes of metaphase chromosomes are studied and compared, most often from root tips mitosis, but in certain favorable groups, for examples, Liliales and Bryophytes, gametophytic karyotypes from the first microspore division can be used (Stebbins, 1971).

As any morphological character of an organism, karyotype is considered more or less similar between individuals of a population. Each species is characterized by its own karyotype (Sharma and Sharma, 1968). Sometimes, karyotypes of different species of a genus may either be much alike (Stebbins, 1971), but the present understanding on karyotypes indicates the presence of remarkable variation in karyotype among individuals in a species (Mascarello and Bolles, 1980) as well as between different species (Raven and Johnson, 1991, 1996). _

Karyotypes are categorized as symmetrical and asymmetrical karyotypes. A symmetrical karyotype is one in which the chromosomes are all approximately the same size, homogenous and have median or sub-median centromeres (Stace, 2000; Stabbins, 1971), whereas in asymmetrical karyotype the chromosomes are heterogeneous and occur either through shifting the centromere position from median to sub-median or terminal, or through accumulation of differences in relative size between the chromosomes of the complement, thus making the karyotype more heterogeneous (Stebbins, 1971).

An asymmetrical karyotype consisting of two sharply distinct size classes of chromosomes, large and short, is called bimodal karyotype (Stebbins, 1971). The origin of bimodal karyotype has been explained in two ways; either they are derived from symmetrical karyotypes of polypolid origin (Darlingthon, 1973) or they are arising from unequal translocation (Stebbins, 1971).

The karyotypic differences are usually observed and compared in terms of differences in absolute size of chromosomes, relative chromosome size, position of centromer, basic number, number and position of the satellites and degree and distribution of heterochromatic regions (Stebbins, 1971).

1.3.5. Evolution of Karyotype

Karyotypes are dynamic. i.e. they change in the course of time because of chromosome rearrangements or numerical. Changes in the chromosome number, chromosome morphology, relative and absolute size of the chromosomes due to numerical or structural changes of chromosomes are the factors that involved in the evolutionary change of chromosomes (Stebbins, 1971).

1.3.5.1. Changes in Chromosome Number and Structure.

Chromosome changes occur from time to time in the cell lineages of all species as a mutational undercurrent constantly adding to the range of variation available for selection (Jones, 1970). The change in the number and sequence of genes or chromosomes is collectively known as Chromosome Aberration (Sinha and Sinha, 1976). As indicated by

Sinha and Sinha, (1976), cosmic radiations, variation in environmental or nutritional factors and certain chemicals can induce chromosome aberrations.

1.3.5.1.1. Chromosome numerical change in karyotype evolution

Comparison of the chromosome count has revealed the presence of considerable variation in chromosome number within a single genus or species (Lewis and Harlan, 1993) and these chromosomal variations are widespread in plants and animals (King, 1993; Rieseberg, 2001). Many of the reports on chromosome numerical variation have shown that a combination of aneuploidy and euploidy can lead to variable numbers of chromosomes in any single cell, organism or race of organisms (Sinha and Sinha, 1976). Some variations in the diploid number in some species of the group have been also attributed to the presence of B-chromosomes (Borin and Santos, 2004).

Aneuploidy means the presence of chromosome number, which is different from a multiple basic chromosome number in one or few chromosomes (Gupta, 2004), which is due to either a loss or gain in chromosome number by less than a complete set (Jackson, 1971). Aneuploids can arise in a number of ways; the most direct of these is by the chromosome non-disjunction (Lewis and John, 1963).The other possible mechanisms are unequal reciprocal translocation (Stebbins, 1950), fusion and fission of chromosomes (Jackson, 1971), mis-division of the centromere (Darlington, 1973) and the incorporation of an extra pair of chromosomes of the regular complements as a result of lagging during cell division (Grant, 1981).

A change in chromosome number, which either decreases or increases the number by a complete genome is often known as euploidy (Jackson, 1971). Euploid is an individual that possesses one or more extra full sets of chromosomes (Gupta, 2004).

Polyploidy is the state where chromosome complements are consisting of three or more basic sets (Ramsey and Schemske, 2002). Polyploidy is extremely common in all the major groups of plants, except the gymnosperms (Lewis, 1980) and has played an

important role in plant evolution (Grant, 1981; Avers, 1980), but rear in animals (Lewis, 1980).

The extent of polyploidy can be revealed only in conjunction with a decision on base-numbers. When there is a single base-number, it is very simple to calculate, but the situation is more difficult where more than one base-number occur, where aneuploidy is common, or where different interpretations of the base-number can be made (Stace, 2000).

Polyploidy can arise naturally in a number of different ways. For examples, due to somatic mutation which results in chromosome doubling in a meristematic cell(s) or the union of unreduced gametes (Sinha and Sinha, 1976; De Wet, 1971).

In terms of time of origin, polyploids are classified into Neopolyploids and Paleopolyploids. Neopolyploid species are those having gametic chromosome numbers that are multiples of the basic diploid chromosome number found in their respective genera. While Paleopolyploid species are ancient, rediploidized polyploids with large basic chromosome numbers (Goldblatt, 1980).

Stebbins (1971) further classified polyploids into Autopolyploids, Allopolyploids and Autoallopolyploids. Autopolyploids are those polyploids with the same chromosome set, arising through multiplication of the complete haploid set of a species (Grant, 1981). Thus, they possess similar genomes. Where as allopolyploids containing genetically different haploid sets of chromosomes or involve different basic sets. The genomes in true allopolyploids are dissimilar and the genomes of a segmental allopolyploid are homeoelogus (Stebbins, 1971). Thus, Segmental allopolyploids are, therefore, intermediate between autopolyploids and allopolyploids (Gupta, 2004).

Polyploidy has been a very important factor in the evolution of certain plant groups and has in the past apparently resulted in the formation of a number of new species and several new genera (Grant, 1981). Thus, it plays important roles in the evolution and

diversification of plants (Soltis *et al.,* 2003). In addition to the above, polyploidy has important roles in the stabilization of several hybrid genotypes, providing a medium by which daughters and parent populations become immediately isolated from each other genetically (De Wet, 1971) and for obtaining fertile hybrids between species and genera (Snitha and Snitha, 1976).

1.3.5.2. B-Chromosomes

B-chromosomes are dispensable supernumerary chromosomes that are not homologous and do not pair with the normal complement (A-chromosomes) (Beukeboom, 1994) and which have irregular and non-mendelian modes of inheritance (Jones, 1995). Most of them are heterochromatic chromosomes composed of repetitive DNA and contain genetically inert DNA (Camacho *et al*., 2000). They present a wide spectrum of variability in their shape, size and stain-ability in comparison to the A-chromosomes (Sharma, 1991). They occur in natural populations of many plant and animal species (Gupta, 2004).

Although B-chromosomes generally do not express major genes, they may affect quantitative characteristics of the carriers especially traits associated with vigor, survival, fecundity and fertility (Camacho *et al.*, 2000) and they give rise to numerical chromosome polymorphism in hundreds of plant and animal species (Jones and Rees, 1982).

1.3.6. Structural Changes

Chromosomes undergo structural changes either spontaneously or artificially under the influence of radiation, chemical mutagens or due to technical faults (Stace, 2000; Griffith *et al*., 2000).

Gupta (2004) the following are the main types of chromosomal structural changes and they constitute the raw material for karyotype repatterning during speciation (Grellhuber and Ehrendorfer, 1988).

1. Deficiency, which involves loss of a part of chromosome that results in loss of chromosome.
2. Duplication, which involves addition of a part of chromosome or involves quantitative change that results in gain of chromosome.
3. Translocation, which involves exchange of segments between non-homologous chromosomes. Robertsonian translocation leads to numerical and structural change without changing the total chromosome number (Stebbins, 1971).
4. Inversion, which involves a reverse order of the genes in a part of chromosome**.** This causes structural changes without changing chromosome number (Stebbins, 1971).

Each of these events can be caused by breakage of DNA double helices in the genome at two or more different locations, followed by rejoining of the broken ends and create a new chromosomal arrangement of genes, different from the gene order of the chromosomes before they were broken (Griffith *et al*., 2000).

1.3.7. The Importance of Karyotypes

The fundamental concept in using karyotypes in systematics is that most species of living organisms show a distinct and constant individuality of their somatic chromosomes and that closely related species have more or less similar chromosomes than those of more distantly related ones (Sharma, 1991).

Chromosome number and other karyotypic data, such as chromosome size, centromeric position, number and sites of secondary constrictions as well as banding pattern can be useful for the assessment of generic or specific relationship (Sinha, 1980) and are also important features used in taxonomic studies (Stace, 1980).

Karyotype has many important practical applications. The knowledge of karyotype is, therefore, useful to address the following points.
1. In addition to morphological and molecular evidences, it can also be of great importance in clarifying the systematic position of a taxon (Takhtajan, 1997), thus

karyotypic analysis gives information about the phylogenic status of species within a group.

2. Karyotype might be important as isolating mechanisms in speciation and have their own evolutionary trends independent of genetic evolution (King, 1993).

3. Differences in karyotype have aided many taxonomic decisions and have provided telling clues in unraveling evolution, for instance in terracing the percentage of hybrids or origin of genomes in polyploidy (Stace, 2000).

4. Studies of karyotypes morphology have led the way to a new and fewer understanding of the systematic relationships within major groups of plants and to complete recognition of the taxonomic system of the group (Stebbins, 1971) as well as karyotypes are used to deduce the relationships between same species (Chen, *et al.*, 2004).

2.0. OBJECTIVES

2.1. General Objective

- To study the cytogenetics of some selected species of afroalpine plants from Bale Mountains in order to fill a gap in chromosome number and Karyotype information.

2.2. Specific Objectives

- To assess the chromosome number of selected plant samples.
- To document new chromosome numbers where chromosome numbers were not previously studied.
- To confirm the reported chromosome numbers of the selected afroalpine plants before.
- To determine the basic chromosome number and ploidy level of the selected afroalpine plants.
- To provide the chromosome description (chromosome formula, ideograms) of some selected afroalpine plants under present study.

3.0. MATERIALS AND METHODS

3.1. Study Area

The Bale Mountains, among the largest mountain massifs in Ethiopia, comprise the largest continuous area above 3000m, in Africa (Fig. 2). They are located in the Southern-eastern of Ethiopian Highlands within the 2470km^2 Bale Mountains National Park (Hillman, 1986). They are one of isolated high mountains in East Africa, mostly of volcanic origin and formed by lava outpouring between 40-25 million years ago. Glaciation has modified the high plateau more recently (20-12000 years B.P), remaining the upper soil and leaving moraines and small lakes behind (Mohr, 1971).

The climate of Bale Mountains varies in different areas according to altitude. The annual rainfall varies from 800 to 1500mm at different altitudes and is characterized by an April-October raining season and a November- March dry season, with warm days and subzero night temperature. Extremes of temperature occur at the highest altitude during dry season (Hillman, 1986, Miehe and Miehe, 1994).

This study is carried on some selected afroalipine plants collected randomly from Bale Mountains National Park, i.e. located between longitudes 39^{o}E and 40^{o}E, and between latitude 6^{o}N and 7^{o}N (Miehe and Miehe, 1994) and it is one of the largest and most diverse national park which is covered with thick forest and grassland (Fig.3).

Fig. 2 Photograph of Bale Mountains

Bale Mountains National Park (BMNP), an area of spectacular afroalpine habitat and moist tropical forest, is the most important conservation area in Ethiopia. It was establish in 1969, following Brow's recommendation to protect the mountain Nyala and Ethiopia wolf. The Park (Fig. 3), therefore, falls within Conservation International's Afro-montane hotspot, and contains the biggest continuous area of Afro-alpine (~1000 km2, 17.5% of all Afro-alpine) and a large proportion of the second largest moist tropical forest in Ethiopia.

The BMNP consists of three main zones-the northern Gaysay montane grasslands between 3000-3400m, the main mountain massif above 2500m and the Southern montane moist evergreen Harenna Forest between 3500-1500m (Hillman, 1986). Within the BMNP there are a number of peaks such as Sanetti plateau (3800-4050m, in the east), Tullu Deemtu (3800-4377m, in the south) and Flat valley (3450m) (Miehe and Miehe, 1993).

There are three major habitat types within BMNP. Namely Afroalpine belt (which is characterized by sparse, short vegetation adapted to low rainfall, heavy frosts, desiccating winds during dry seasons (Miehe and Miehe, 1994), Ericaeous belt (above the treeline, between 3400-3800m, covered by ericaeous vegetation), (Miehe and Miehe, 1993) and the montane Grasslands (dominated by swamps grasses and sedges, lower bushes) (Menassie Gashaw and Masresha Fetene, 1996).

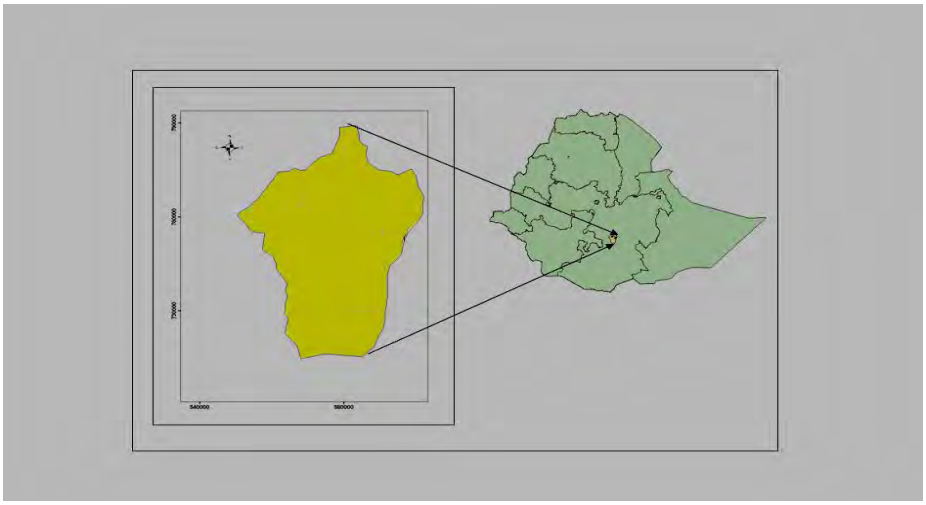

Fig. 3 The map of BMNP

3.2. Plant Materials

The plant specimens (Table.1; Fig. 1) were used for the cytogenetical studies were collected randomly from Bale Mountains at altitudes of between 1000- 4350masl and grown in the greenhouse at Addis Ababa University. Many of the collected specimens were unable to reestablish in the greenhouse because of the different climate of the green house. Only specimens of ten species were used in the present cytological study.

Identification of some of the plant specimens was made in the field and others were further identified at the National Herbarium, Addis Ababa University, where the Voucher specimens are deposited. Nomenclature for the taxa examined was followed the published volumes of Flora of Ethiopia and Eritrea (Hedberg and Edwards, 1989, Edwards *et al*., 1995).

Table. 1 Scientific name, code, Family name, altitude range and habit of selected afroalpine plants under present study.

NO	Taxon	Code	Family	Altitude(masl)	Habit
1	*Centella asiatica*	4B	Apiaceae	1000-3200	Perennial herb
2	*Cotula cryptocephala* .	17C	*Asteraceae*	2800-3750	Perennial herb
3	*Senecio nanus*	1C	*Asteraceae*	3250-4100	Perennial herb
4	*Crassula alsinoides*	1E	Crassulaceae	1500-3100	Perennial herb
5	*Geranium arabicum*	3F	Geraniaceae	1300-3650	Perennial herb
6	*Lobelia rhychopetalum*	16G	Lobeliaceae	3000-4350	Perennial shrub
7	*Plantago major*	58	Plantaginanceae	1300-2400	Perennial herb
8	*Rumex nepalensis*	6A	Polygonaceae	1200-3900	Perennial herb
9	*Ranunculus multifidus*	57	Ranunculaceae	1200-3800	Perennial herb
10	*Ranunculus oreophytus*	14B	Ranunculaceae	2500-4000	Perennial herb

Fig.1a-i Morphology of selected afroalpine plant species incorporated in the present study.a-*Centella asiatica* L. Urban; b-*Cotula cryptocephala*; c-*Senecio nanus*; d-*Crassula alsinoides*; e-*Geranium arabicum*; f-*Lobelia rhychopetalum*; g-*Plantago major*; h-*Rumex nepalensis*; i-*Ranunculus multifidus* and j-*Ranunculus oreophytus*.

3.3. Methods

3.3.1. Somatic Chromosome Preparation

Somatic chromosome preparations were made from root tip meristems obtained directly from plants transplanted in the greenhouse at AAU. The method used was as described in Kifle Dagne and Heneen (1992).

The tips of actively growing and healthy roots (3-4cm long) were cleaned of soil particles subjected to cold treatment by keeping them in ice water for at least 24hrs or treated with colchicine for at least 3 hours. The root tips were then fixed in fresh ethanol-acetic acid (v/v 3:1) for at least 24 hr at 4°C following Stedje (1996). After briefly rinsing in distilled water three to four times, enzyme maceration was performed in 4% cellulase + 4% pectinase solution for about 1hr at 37°C (Kifle Dagne and Heneen, 1992). When the roots were macerated, the lower1-2 mm meristematic tips detached from the root. The root tips were rinsed in distilled water after decanting the enzymes.

3.3.2. Slide Preparation (Air-Dry Technique).

The detached lower ends of the macerated root tips were pipetted onto glass slide and the water was removed by toughing the edge of the water drops using filter paper. Depending on the sizes, from one to the several root tips were used per slide. The root tips were then mashed in a few drops of fresh fixative (3:1, ethanol: glacial acetic acid, v/v) using a flat end needle. Then cells were spread by strongly blowing on the slide. The slides were allowed to air dry under room temperature (Kifle Dagne and Heneen 1992).

Permanent slides with better chromosome preparation were made by staining air dry preparation in Giemsa stain in Sorensen's phosphate buffer (pH = 6.8) (half an hours) followed by rinsing in the same buffer, air drying and mounting in DPX mountant. The permanent slides were examined under the light microscope and cells with spread chromosomes were photographed for determination of chromosome numbers and karyotype.

3.3.3. Chromosome Count

Chromosomes counts were made under the microscope and from photomicrographs. Count for each species were made from at least eight to nine well-spread metaphase in intact cells. Here, the highest chromosome number was scored for each species. Cells with a good spread of chromosomes were photographed using a camera fitted microscope at a magnification of 1000 (i.e. 10x eyepiece and 100x objective).

Table. 2. Plant taxa studied, number of potted plants analyzed and number of metaphase plates analyzed per species

Taxon	Number of potted plants analyzed	Number of metaphase plates analyzed per each species
Centella asiatica	7	8-9
Cotula cryptocephala	8	8-9
Senecio nanus	8	8-9
Crassula alsinoides	8	8-9
Geranium arabicum	8	8-9
Lobelia rhychopetalum	8	8-9
Plantago major	7	8-9
Rumex nepalensis	8	8-9
Ranunculus multifidus	8	8-9
Ranunculus oreophytus	8	8-9

3.3.4. Karyotype Analysis

Karyotypes of somatic chromosomes were determined for three species only, *Senecio nanus*, *Plantago major* and *Ranunculus oreophytus.* From a set of mitotic metaphase plates of each species, a representative chromosome spread was selected and scanned into a computer and the total length and arm lengths of each chromosome in pixel's per cm, were measured using Micro-measure computer software Version 3.3.

Karyotype formulae were determined on selected enlarged prints by carefully measuring the length of chromosome arms. Arm ratios of each chromosome were calculated by dividing the length of the long arm to the length of short arm. The karyotypes were then constructed from pictures of the metaphase plates by cutting and arranging homologous chromosomes into pairs using centromeric position (arm ratio) and chromosome size as criteria.

The centromeric index was calculated as the long: short arm ratio to classify chromosomes following the system of Levan *et al.* (1964): m = metacentric (r = 1·00–1·69), sm = submetacentric (r = 1·70–2·99), st = subtelocentric (r = 3·00–6·99).

3.3.5. Chromosomes length

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

Normalized length of a chromosome refers to its relative length to the total complement length of the haploid complement. The length of each chromosome was then expressed as a percentage of the total haploid complement length which is calculated by dividing the length of each chromosome by the total length of the haploid chromosomes set and then multiplied by 100. The resultant percentage figures were then multiplied by 10 to avoid decimals and produce units of normalized length which could be used to compare individual cells and species (Bogart, 1974).

The standards of Stebbins (1938) were used for distinguishing the chromosomes; <2.0 µm, small; 2.0 - 5.0 µm, Medium-small; 5.0 - 9.0 µm, medium-large; >9.0 µm, Large.

Similarly, we were used Bogart's (1974) scheme of chromosomes classification to define chromosomes as small, < 80 units; intermediate, 80 – 100 units; and large, ≤100 units.

4.0. RESULTS

4.1. Chromosome count

The results of somatic chromosome counts showed that *Centella asiatica* has the somatic chromosome number 2n = 20 and it is diploid; *Cotula cryptocephala* has the somatic chromosome number 2n = 72 and it is nanoploid; *Senecio nanus* has the somatic chromosome number 2n = 40 and it is tetraploid; *Crassula alsinoides* is either pentaploid or octaploid with 2n = 56; *Geranium arabicum* has the somatic chromosome number 2n = 28 and it is diploid; *Lobelia rhychopetalum* has the somatic chromosome number 2n = 28 and it is tertraploid; *Plantago major* with the smallest chromosome number , 2n = 12 and it is diploid; *Rumex nepalensis* has a somatic chromosome number of 2n = 18 and it is a diploid; and chromosome number 2n = 32 was found for both *Ranunculus multifidus* and *Ranunculus oreophytus* and are both tertraploid (Table. 3; Fig. 4).

Table 3. The somatic chromosome number (2n), basic numbers and level of ploidy of the investigated taxa

Taxon	Previously reported chromosome number (2n)	Present finding of chromosome number (2n)	Basic chromosome number	Level of ploidy
Centella asiatica ♥	__	20	10	2x (diploid)
Cotula cryptocephala ♠	80	72	8	9x (nanoploid)
Senecio nanus ♣	40	40	10	4x (tetraploid)
Crassula alsinoides ♥	__	56	7	8x (octaploid)
Geranium arabicum ♣	28	28	14	2x (diploid)
Lobelia rhychopetalum ♣	28	28	7	4x (tetraploid)
Plantago major ♥	__	12	6	2x (diploid)
Rumex nepalensis ♥	__	18	9	2x (diploid)
Ranunculus multifidus ♣	32	32	8	4x (tetraploid)
Ranunculus oreophytus ♣	32	32	8	4x (tetraploid)

Key: - indicates ♠ Different record, ♥ First record, ♣ Second count/ record

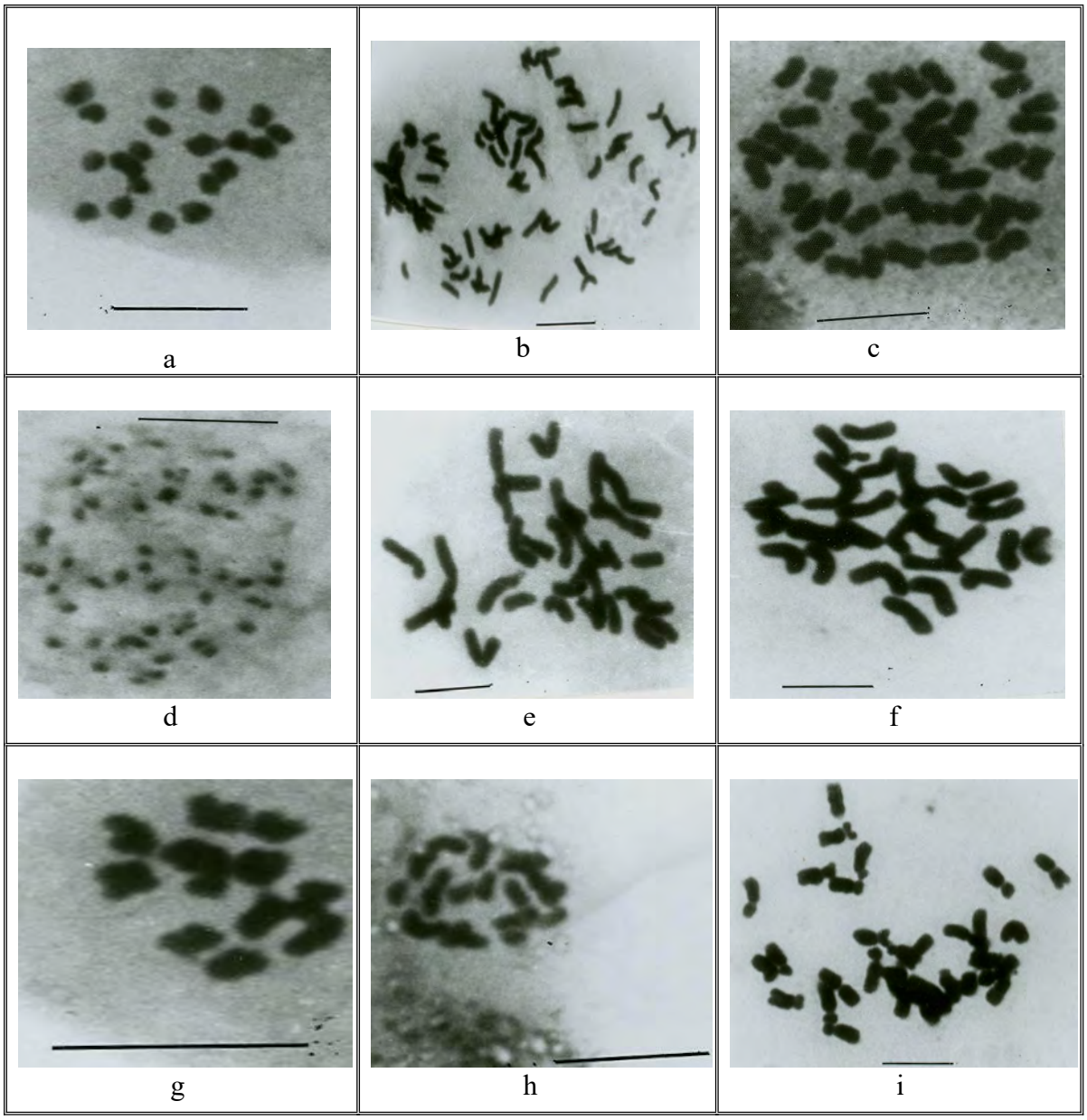

Fig.4 (a-i). Photopmicrographs of metaphase chromosome plates. a-*Centella asiatica* L. urban (2n = 20); b-*Cotula cryptocephala*(2n = 2); c-*Sencio nanus* (2n = 40); d-*Crassula alsinoides* (2n = 56); e-*Geranium arabicum* (2n = 28); f-*Lobelia rhychopetallum* (2n = 28); g-*Plantago major* (2n =12); h-*Rumex nepalensis* (2n =18); i-*Ranunculus multifidus* (2n = 32) and j-*Ranunculus oreophytus* (2n= 2). Scale bars =5 µm

4.2 KARYOTYPIC ANALYSIS

The karyotypic analysis showed that *Sencio nanus* has a karyotype, consisting of 20 pairs of metacentric chromosomes (Fig.5; Appendix. 1). The karyotype formula of this species is given as 40 m. The analysis of chromosome size showed that the chromosome lengths of *Sencio nanus* range is between 3.0 - 5.0 µm (Appendix. 4), which is medium-small or small.

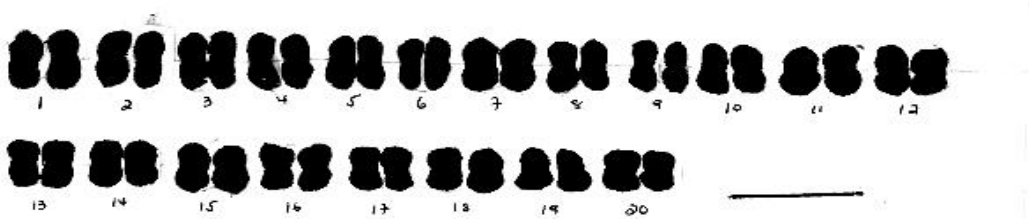

Fig.5. Diploid ideograms of *Senecio nanus*

Plantago major like *Senecio nanus* has karyotype that consists of 6 pairs of metacentric chromosomes with a karyotypic formula of 2n = 12m (Fig.6; Appendix. 2). The chromosome lengths of *Plantago major* lie in a range between 2.28 – 2.5 µm (Appendix. 4), which is medium-small according to or large.

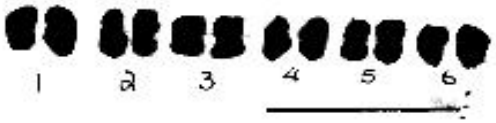

Fig.6. Diploid ideograms of *Plantago major*

Ranunculus oreophytus has karyotype with the karyotype formula of this species is 2n = 8m + 8sm + 16st (Fig.7; Appendix. 3). The analysis of chromosome size showed that the chromosome lengths of *Ranunculus oreophytus* range in between 7.80 - 11.35 µm (Appendix. 4), 9 of which are medium-large (7.8 – 8.75 µm) and 23 large (9.13 – 11.35 µm or all are small.

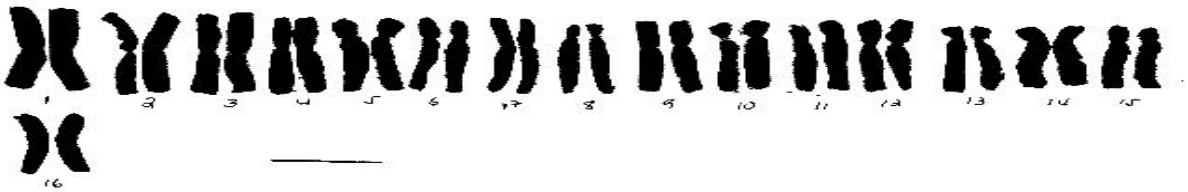

Fig.7 Diploid ideograms of *Ranunculus oreophytus*

5.0. DISCUSSIONS

Due to the limitations rendered by the small size of the chromosomes and lack of significant morphological differentiation between the chromosomes of most of the species, it was not possible to give detailed cytogenetical characterization of most of the species. It has to be noted that some chromosomal morphological features are better observed under the microscope than using their photomicrographs.

Well-condensed and spread chromosomes usable for karyotypic analysis could not be obtained in most of the species under this study due to inherent and technical problems. Although it has not been possible to construct karyotypes for most of the studied species, chromosome counts have been made for all of the ten species studied, namely *Centella asiatica* L. Urban*, Cotula cryptocephala, Senecio nanus*, *Crassula alsinoides, Geranium arabicum, Lobelia rhychopetallum, Plantago major, Rumex nepalensis*, *Ranunculus multifidus* and *Ranunculus oreophytus*.

Chromosome counts obtained for five of the species, *Senecio nanus*, *Geranium arabicum, Lobelia rhychopetallum, Ranunculus multifidus* and *Ranunculus oreophytus* are conformations of earlier reports made by different authors (Hedberg and Hedberg, 1977; Bennett and Smith, 1991; Lammers, 1992; Thulin, 1970). Regarding *Cotula cryptocephala,* the chromosome count obtained in this study is different from what have been reported earlier (Hedberg and Hedberg, 1977). To date, there is no report on the chromosome number of *Centella asiatica, Crassula alsinoides, Plantago major* and *Rumex nepalensis*. So their chromosome numbers are, therefore, reported here for the first time.

5.1. *Centella asiatica*

The result of 2n = 20 found for this species in the present study supports the earlier hypothesis of basic number of x = 10 for the family Apiaceae (Bennett and Smith, 1991). This species is, therefore, diploid with 2n = 2x = 20 (Fig. 4a).

5.2. *Cotula cryptocephala*

The present finding is at variance with a previous report on the chromosome number of this species by Hedberg and Hedberg (1977). Thus chromosome number (2n = 72) is presented here for the first time (Table.3; Fig.4b).

Although the count of 2n = 80 for *Cotula cryptocephala* by Hedberg and Hedberg (1977) was the first record for this species, in the present study the somatic chromosome number of this species was found to be 2n = 72. Therefore, from this we can say that the species exists in more than one ploidy levels and thus there exist more than one cytotypes of *Cotula cryptocephala*.

The present finding is in agreement with previously reported basic chromosome number of genus *Cotula* (x = 8) as reported by Hedberg and Hedberg (1977) and for the family by Thulin (1970) and Bennett and Smith (1991). From these observations we can concluded that this species is a nanoploid with the basic chromosome number of x = 8.

5.3. *Senecio nanus*

The karyological examination of *Senecio nanus* revealed 2n = 40 (Table.3; Fig. 4c) and suggested that it is a tetraploid species having a basic chromosome number x = 10. This chromosome count agrees with that given by Hedberg and Hedberg (1977). Therefore, our count definitely confirms the earlier chromosome number reported for this species.

The karyotype of *Senecio nanus* with 2n = 40 consists of 40 metacentric. The karyotypic formula of this species is, therefore, given as 40m. This is the first work on karyotype morphology of *Senecio nanus*. *Senecio nanus* was found to have symmetrical karyotype because its all chromosomes are grouped in one distinct size class or homogenous type of chromosomes with only slight size variation within them (Fig.5). Similarly, all chromosomes are belonging to one group based on the centromeric position, metacentric type and therefore *Senecio nanus* has a symmetrical karyotype as mentioned in Stebbins (1971).

Although the karyotype of this species seems composed of uniform or homogeneous chromosomes, all with metacentric centromeric position the length of the chromosomes vary between 3.00 and 5.0 µm (Appendix. 4). The ratio of the largest to the smallest chromosomes is 1.66. All chromosomes in the complement belong to one group, the Medium-small range of Stebbins (1938) varying between 2.00-5.00µm and also to the small category of Bogart (1974), the total haploid complement falls below 8% or chromosomes have a value of less that 80 units.

5.4. *Crassula alsinoides*

Even though Hedberg and Hedberg (1977) and Randle *et al*. (2005) have studied few species of *Crassula*, no such record (2n = 56) on the chromosome number available in the literature. So the chromosome number of 2n = 56 of this species (Table.3; Fig.4d) is presented for the first time for this species.

As already noted by Hedberg and Hedberg (1977) and Randle *et al*. (2005), the genus *Crassula* is characterized by two basic chromosome numbers of x = 7 and 8. Based on this, the present count of 2n = 56 fits with ploidy level of either 7x when basic chromosome number of x = 8 is considered or ploidy level of 8x when basic chromosome number of x = 7. The former ploidy level, 7x with basic chromosome number of x = 8 is a new report that was not mentioned for the genus in any of literatures before.

It has been reported that the genus *Crassula* has ploidy levels 2x, 4x, 5x, 6x, 8x and 12x (Hedberg and Hedberg, 1977). So, based on this information, our chromosome count of 2n = 56 is more likely indicates that *Crassula alsinoides* has a ploidy level of 8x rather than 7x and this may probably suggest a possible basic chromosome numbers of x = 7, and if it is so this species is an octaploid plant.

5.5. *Geranium arabicum*

Table 3 and Fig.4e show that the chromosome number of this species is 2n = 28. This result is in agreement with previous study on the same species made by Hedberg and

Hedberg (1977). Therefore, our count confirms the earlier observation made by Hedberg and Hedberg (1977).

Previous reports on basic chromosome number and ploidy level of this species (Hedberg and Hedberg, 1977) showed that this species is diploid with the basic chromosome number of x = 14. From our finding and earlier reports available in the literatures on the cytology of this particular species, it is believed that *Geranium arabicum* is represented by ploidy level of 2x with basic chromosome number of x = 14 and therefore we assume that this is a diploid species.

5.6. *Lobelia rhychopetalum*

The chromosome count for *Lobelia rhychopetalum* obtained is 2n = 28 (Table.3; Fig.4f). The same number has been reported by Hedberg and Hedberg, (1977). Therefore, the present count confirms the earlier chromosome number report for this species.

Since the basic chromosome numbers of the genus *Lobelia* are x = 6, 7, 8, 10 and 13 (Bennett and Smith, 1991**;** Lammers, 1992; Hedberg and Hedberg, 1977) with ploidy levels of 2x, 3x, 4x (Bennett and Smith, 1991; Hedberg and Hedberg, 1977), our finding has shown that the present material is a tetraploid cytotype having ploidy level of 4x with a basic chromosome number x = 7. This cytological information is supported by the works of both Bennett and Smith (1991) and Hedberg and Hedberg (1977).

5.7. *Plantago major*

The present study of the somatic chromosomes of *Plantago major* revealed a diploid karyotype with 2n = 12 (Table.3; Fig.4g). We could not find any previous report on chromosome number of this species. However, in some literatures it is indicated that most species of genus *Plantago* have the basic chromosome number of x = 6 (Bennett and Smith, 1991). Thus, it is apparent that this species is diploid with a basic chromosome number of x = 6.

The analysis of the karyotype of *Plantago major* showed that, this species is characterized by a unimodal or a symmetrical type of karyotype in which the whole chromosomes complements possess r-values between 1.0 - 1.69 (Levan *et al*., 1964). On the basis of arm ratio, all chromosomes are grouped as metacentrics. Therefore, its karyotypic formula is written as 2n = 12m.

Even though the chromosome lengths of this species vary with minor differences in the range of 2.28 - 2.5µm and the sizes of the chromosomes of *Plantago major* are consistently similar, nearly all belong to the Medium-large of Stebbins (1938) because their chromosomes lengths lie in a range between 2.00 µm and 5.00µm. On the other hand, according to Bogart (1974) the chromosomes of this species belong to the large chromosomes category, because the chromosomes have a value of greater than 100 units or each chromosome contributes above 10% greater than of the total haploid complement (Appendix 8).

5.8. *Rumex nepalensis*

Chromosome number of 2n = 18 was found in the present study (Table.3; Fig.4h). We could not find any chromosomal data recorded for this species in the literature. It may be assumed that the present report is the first for this species.

Although there is no previous work published on the cytology of this particular species so far, the family Polygnanceae has been reported to have the basic number of x = 6, 7, 8, 9, 13 and 17 with ploidy of 4x (Hedberg and Hedberg, 1977) and x = 7, 10, 11 with 2x and 4x ploidy levels (Bennett and Smith,1991). Based on these information, it is likely that, this species is diploid based on the basic chromosome number of x = 9 indicated for the genus by Hedberg and Hedberg (1977).

5.9. *Ranunculus multifidus*

Thulin (1970) indicated that *Ranunculus multifidus* has 2n = 32 with the basic chromosome number x = 8 which is in agreement with our present report of 2n = 32 (Table.3; Fig.4i).

The present finding also agrees with the reports on the basic chromosome number of x = 8 which was suggested by Thulin (1970) and Hedberg and Hedberg (1977). Therefore, the available count for this species confirms the previous record on basic chromosome number. On the basis of x = 8, this species would be tetraploid (4x).

5.10. *Ranunculus oreophytus*

The somatic chromosome number obtained for this species (2n = 32) coincides with the prior determination (Hedberg and Hedberg, 1977) and the basic chromosome number x = 8 observed in this species is also the same as that by Hedberg and Hedberg (1977) (Table.3; Fig.4j). Our present finding can be taken as a conformation on the earlier observation on the cytology of *Ranunculus oreophytus*. This species is, therefore, thought be a tetraploid plant having a ploidy level of 4x with the basic chromosome number of x = 8.

From the present study on the karyogram of this species, the chromosomes vary in the position of the centromere and the arm length (Levan *et al*., 1964). According to the centromeric positions and arm ratio, the chromosomes are, therefore, grouped into 8 metacentrics (chromosomes pairs 1, 2, 3, 16), 8 sub-metacentrics (chromosomes pairs 4, 7, 9, 12) and 16 sub-telocentrics chromosomes (chromosomes pairs 5, 6, 8, 10, 11, 13, 14, 15) (Fig.6; Appendix 3). Therefore, the karyotype formula of this species is written as 2n = 32 = 8m+ 8sm + 16st.

Chromosomes of this species are unequal in size and vary in centromeric positions (m, sm and st) and are, thus heterogeneous. Therefore, the chromosomes of *Ranunculus oreophytus* is characterized as an asymmetrical karyotype according to Stebbins (1971) and Stace (2000)

Even if the present karyotype analysis of *Ranunculus oreophytus* indicated that there is a slight variation in the overall chromosome size, the karyotype of this species has two remarkable types of chromosomes, Medium-large (which range in length from 7.80 to 8.75µm) and large chromosomes (whose lengths range from 9.13 to 11.35µm) based on

the chromosomes classification made by Stebbins (1938). Meanwhile among the large chromosomes 10, 6 and 7 chromosomes are subtelocentric, submetacentric and metacentric, respectively. Moreover, 6 subtelocentric, 2 submetacentric and 1 metacentric chromosomes have medium-small size (Stebbins, 1938).

On the other hand, the chromosomes of this species are defined as being small according to the chromosomes classification developed by Bogart (1974) since the chromosomes have a value of less than 80 units i.e. 8% less than of the total haploid complement (Appendix 10).

6.0. CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

Cytologically, the Afroalpine plants of Bale Mountains, Ethiopia have not been investigated widely; only very few species are examined for chromosome number.

The present study involves an original (first) chromosome count for *Centella asiatica* L. Urban, *Crassula alsinoides, Plantago major* and *Rumex nepalensis*, a conformation of the previous reports for *Senecio nanus*, *Geranium arabicum, Lobelia rhychopetalum*, *Ranunculus multifidus* and *Ranunculus oreophytus* different chromosome count has made for *Cotula cryptocephala* (2n = 72), than previously reported.

As karyotypic analysis revealed that, both *Senecio nanus* and *Plantago major* have a symmetrical karyotype consisting of 20 pairs and 6 pairs of metacentric chromosomes, respectively. Whereas *Ranunculus oreophytus* showed asymmetrical karyotype with 4 pairs of metacentric, 4 pairs of sub-metacentric and 8 pairs of sub-telocentric chromosomes. Size variation within a complement can be seen in *Senecio nanus*, *Plantago major* and *Ranunculus oreophytus*.

6.2. RECOMMENDATIONS

Cytologically, still the Afroalpine plants are poorly understood and almost all Afroalpine plants of Ethiopia in general are not yet examined. More studies are, therefore, necessary to be conducted in the future.

The present study in fact has focused on a limited number of Afroalpine plants from Bale Mountains, thus it is recommended that further studies should be carried out on the other members of this group with different altitudinal and environmental distribution to obtain reliable and comprehensive data.

In this study we were unable to give detailed karyotypic analysis to most species under the present study. Therefore, it is better to carry out further cytological studies (C-banding, Silver staining, FISH) in the future.

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7.0. APPENDICES

Appendix 1. Chromosome measurements, an arm ratio and position of the centeromere in *Senecio nanus*
Mag: 2200

Image resolution: 78.74 pixels per cm

Marking order	Rank	Length each	Long arm	Short arm	Arm Ratio (L/S)	Centromeric position
12	1	3.99	2.01	1.98	1.01	m
7	2	3.93	2.06	1.87	1.10	m
10	3	3.63	2.07	1.55	1.33	m
9	4	3.57	2.00	1.57	1.27	m
1	5	3.52	1.92	1.60	1.20	m
23	6	3.47	1.80	1.67	1.07	m
16	7	3.37	1.73	1.63	1.06	m
4	8	3.36	1.74	1.62	1.08	m
25	9	3.35	1.76	1.60	1.10	m
31	10	3.34	1.81	1.53	1.19	m
35	11	3.33	1.88	1.45	1.30	m
13	12	3.28	1.77	1.52	1.17	m
8	13	3.25	1.99	1.27	1.57	m
29	14	3.23	1.68	1.55	1.08	m
15	15	3.19	1.72	1.47	1.17	m
40	16	3.18	1.67	1.52	1.10	m
5	17	3.18	1.91	1.27	1.50	m
11	18	3.17	1.59	1.58	1.01	m
19	19	3.17	1.83	1.34	1.36	m
32	20	3.17	1.70	1.46	1.17	m
24	21	3.16	1.61	1.55	1.03	m
34	22	3.15	1.68	1.47	1.14	m
27	23	3.15	1.61	1.54	1.05	m
26	24	3.13	1.81	1.32	1.38	m
21	25	3.11	1.78	1.33	1.34	m
33	26	3.11	1.75	1.35	1.30	m
36	27	3.07	1.72	1.35	1.27	m
20	28	3.03	1.59	1.44	1.10	m
22	29	3.00	1.63	1.36	1.20	m
17	30	2.99	1.63	1.36	1.20	m
39	31	2.95	1.50	1.45	1.03	m
6	32	2.93	1.47	1.46	1.01	m
37	33	2.86	1.43	1.43	1.00	m
28	34	2.81	1.65	1.16	1.43	m
38	35	2.81	1.45	1.36	1.06	m
18	36	2.77	1.51	1.27	1.19	m
14	37	2.69	1.44	1.25	1.16	m
30	38	2.69	1.42	1.27	1.12	m
3	39	2.63	1.36	1.27	1.08	m
2	40	2.62	1.36	1.26	1.08	m

Appendix 2. Chromosome measurements, an arm ratio and position of the centeromere in *Plantago major*

Mag: 2200

Image resolution: 37.80pixels per cm

marking order	Rank	Length each	Long arm	Short arm	Arm Ratio (L/S)	Centromeric position
3	1	4.41	2.44	1.97	1.23	m
4	2	4.35	2.33	2.02	1.16	m
7	3	4.25	2.17	2.08	1.05	m
11	4	4.12	2.13	1.99	1.07	m
12	5	4.05	2.15	1.90	1.13	m
6	6	3.99	2.05	1.94	1.05	m
10	7	3.93	1.99	1.94	1.03	m
2	8	3.89	2.22	1.67	1.32	m
9	9	3.64	2.08	1.56	1.33	m
1	10	3.62	1.82	1.80	1.01	m
8	11	3.37	1.84	1.53	1.20	m
5	12	3.15	1.75	1.40	1.25	m

Appendix 3. Chromosome measurements, an arm ratio and position of the centeromere in *Ranunculus oreophytus*.

Mag: 2200

Image resolution: 78.74 pixels per cm

marking order	Rank	Length each	Long arm	Short arm	Arm Ratio (L/S)	Centromeric position
2	1	9.25	4.78	4.47	1.07	m
4	2	9.06	4.68	4.38	1.07	m
3	3	8.92	4.69	4.22	1.11	m
21	4	8.54	4.34	4.20	1.03	m
28	5	8.52	4.49	4.03	1.12	m
6	6	8.33	4.85	3.48	1.40	m
9	7	8.11	5.12	2.99	1.71	sm
20	8	8.07	5.30	2.77	1.91	sm
11	9	8.01	6.08	1.93	3.15	st
32	10	7.95	6.04	1.91	3.17	st
30	11	7.75	5.84	1.91	3.05	st
25	12	7.70	5.92	1.78	3.33	st
26	13	7.70	5.25	2.45	2.14	sm
15	14	7.68	4.95	2.73	1.81	sm
14	15	7.65	5.84	1.81	3.23	st
19	16	7.65	5.76	1.88	3.06	st
31	17	7.61	4.82	2.79	1.73	sm
10	18	7.60	5.44	2.16	2.51	sm
1	19	7.54	5.73	1.81	3.17	st
18	20	7.53	5.75	1.78	3.22	st
13	21	7.37	6.20	1.17	5.30	st
24	22	7.32	5.84	1.48	3.95	st
29	23	7.26	4.65	2.61	1.78	sm
12	24	7.26	4.63	2.63	1.76	sm
23	25	6.96	5.78	1.18	4.91	st
5	26	6.86	5.17	1.69	3.07	st
22	27	6.73	5.46	1.28	4.28	st
27	28	6.70	5.08	1.63	3.12	st
17	29	6.67	5.46	1.21	4.50	st
8	30	6.64	5.12	1.52	3.36	st
7	31	6.48	3.47	3.01	1.15	m
16	32	5.20	2.94	2.25	1.31	m

Appendix 4. Chromosomes lengths and types of *Senecio nanus*

Chromosome number	Total length (um)	Chromosome type (Stabbins, 1938)
1	3.75	Medium-small
2	3.75	Medium-small
3	3.75	Medium-small
4	4.38	Medium-small
5	3.75	Medium-small
6	3.75	Medium-small
7	4.38	Medium-small
8	4.38	Medium-small
9	4.38	Medium-small
10	3.75	Medium-small
11	3.75	Medium-small
12	3.75	Medium-small
13	3.75	Medium-small
14	5.00	Medium-small
15	3.75	Medium-small
16	3.75	Medium-small
17	3.75	Medium-small
18	3.75	Medium-small
19	3.75	Medium-small
20	3.75	Medium-small
21	3.75	Medium-small
22	3.75	Medium-small
23	3.75	Medium-small
24	3.75	Medium-small
25	3.75	Medium-small
26	3.00	Medium-small
27	3.75	Medium-small
28	3.75	Medium-small
29	3.25	Medium-small
30	3.75	Medium-small
31	3.75	Medium-small
32	3.75	Medium-small
33	4.38	Medium-small
34	4.38	Medium-small
35	3.00	Medium-small
36	3.75	Medium-small
37	3.75	Medium-small
38	3.75	Medium-small
39	3.75	Medium-small
40	3.75	Medium-small

Appendix 5. Chromosomes lengths and types *Plantago major*

Chromosome number	Total length (um)	Chromosome type (Stabbins, 1938)
1	2.50	Medium - Small
2	2.48	Medium - Small
3	2.43	Medium - Small
4	2.40	Medium - Small
5	2.35	Medium - Small
6	2.33	Medium - Small
7	2.31	Medium - Small
8	2.31	Medium - Small
9	2.30	Medium - Small
10	2.30	Medium - Small
11	2.28	Medium - Small
12	2.28	Medium - Small

Appendix 6. Chromosomes lengths and types of *Ranunculus oreophytus*.

Chromosome number	Total length (um)	Chromosome type (Stabbins, 1938)
1	9.80	Large
2	11.35	Large
3	11.25	Large
4	11.20	Large
5	8.75	Medium-large
6	10.63	Large
7	8.00	Medium-large
8	10.00	Large
9	8.13	Medium-large
10	10.5	Large
11	9.25	Large
12	9.80	Large
13	11.00	Large
14	10.60	Large
15	8.10	Medium-large
16	7.80	Medium-large
17	9.13	Large
18	9.20	Large
19	8.13	Medium-large
20	9.65	Large
21	9.94	Large
22	8.70	Medium-large
23	9.65	Large
24	9.94	Large
25	9.38	Large
26	10.30	Large
27	8.75	Medium-large
28	8.75	Medium-large
29	9.25	Large
30	10.00	Large
31	9.25	Large
32	10.63	Large

Appendix 7. Analysis of haploid chromosome complement of *Senecio nanus*

Marking order	Length each	Normalized length	Chromosomes type (Bogart, 1974).
12	3.99	63	Small
7	3.93	62	Small
10	3.63	57	Small
9	3.57	57	Small
1	3.52	56	Small
23	3.47	55	Small
16	3.37	53	Small
4	3.36	53	Small
25	3.35	53	Small
31	3.34	53	Small
35	3.33	53	Small
13	3.28	52	Small
8	3.25	52	Small
29	3.23	51	Small
15	3.19	51	Small
40	3.18	50	Small
5	3.18	50	Small
11	3.17	50	Small
19	3.17	50	Small
32	3.17	50	Small
24	3.16	50	Small
34	3.15	50	Small
27	3.15	50	Small
26	3.13	50	Small
21	3.11	49	Small
33	3.11	49	Small
36	3.07	49	Small
20	3.03	48	Small
22	3.00	47	Small
17	2.99	47	Small
39	2.95	47	Small
6	2.93	46	Small
37	2.86	45	Small
28	2.81	44	Small
38	2.81	44	Small
18	2.77	44	Small
14	2.69	43	Small
30	2.69	43	Small
3	2.63	42	Small
2	2.62	42	Small

Total for set: 126.29 Total haploid for set: 63.15

Appendix 8. Analysis of haploid chromosome complement of *Plantago major*

Marking order	Length each	Normalized length	Chromosomes type (Bogart, 1974).
12	4.41	188	Large
7	4.35	186	Large
10	4.25	181	Large
9	4.12	176	Large
1	4.05	173	Large
23	3.99	170	Large
16	3.93	168	Large
4	3.89	166	Large
25	3.64	156	Large
31	3.62	155	Large
35	3.37	144	Large
13	3.15	135	Large

Total for set: 46.8

Total haploid for set: 23.4

Appendix 9. Analysis of haploid chromosome complement of *Ranunculus oreophytus*.

Marking order	Length each	Normalized length	Chromosomes type (Bogart, 1974).
2	9.25	76	Small
4	9.06	75	Small
3	8.92	74	Small
21	8.54	70	Small
28	8.52	70	Small
6	8.33	69	Small
9	8.11	67	Small
20	8.07	67	Small
11	8.01	66	Small
32	7.95	66	Small
30	7.75	64	Small
25	7.70	63	Small
26	7.70	63	Small
15	7.68	63	Small
14	7.65	63	Small
19	7.65	63	Small
31	7.61	63	Small
10	7.60	63	Small
1	7.54	62	Small
18	7.53	62	Small
13	7.37	61	Small
24	7.32	60	Small
29	7.26	60	Small
12	7.26	60	Small
23	6.96	57	Small
5	6.86	57	Small
22	6.73	56	Small
27	6.70	55	Small
17	6.67	55	Small
8	6.64	55	Small
7	6.48	53	Small
16	5.20	43	Small

Total for set: 242.62

Total haploid for set: 121.31

Appendix 10. Summery on the size and types of chromosomes of *Senecio nanus*, *Plantago major* and *Ranunculu oreophytus*.

Species	Size. Min.-Max.	Types (Stebbins, 1938)	Types (Bogart, 1974)
Senecio nanus	3.0 - 5.0 µm	Medium-small (40)	Small (40)
Plantago major	2.28 - 2.5 µm	Medium-small (12)	Large (12)
Ranunculus oreophytus	7.8 - 8.75 µm 9.13 - 11.35 µm	Medium-large (9) Large (23)	Small (32)

Appendix 11. Summery on the analysis of haploid chromosome complements of *Senecio nanus*, *Plantago major* and *Ranunculu oreophytus*

Species	Total haploid complement	Types (Bogart, 1974)
Senecio nanus	42 - 63	Small
Plantago major	135 - 186	Large
Ranunculus oreophytus	43 – 76	Small

DECLERATION

I, the under signed, declare that this thesis is my original work. It has never been submitted in any institution and that all sources of materials used for thesis have been duly acknowledged.

Name: Teshome Tesfaye Desta

Signature: _____

Date: _____

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

Name	Signature
1. Dr. Kifle Dagne	_____
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