ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

CYTOGENETIC STUDY OF SOME SPECIES OF *SWERTIA* L. (GENTIANACEAE) FROM ETHIOPIA

MULUGETA KEBEDE TESISSA

Addis Ababa, May 2000.

Cytogenetic Study of Some Species of *Swertia* L. (Gentianaceae) from Ethiopia

A Thesis Submitted to the

School of Graduate Studies

Addis Ababa University

In Partial Fulfillment of the Requirement for the Degree of Master of Science in Biology (***Applied Genetics***)

by

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Approved by Examining Board:

Prof. Abdel-Rahem Tawfeek, Examiner	Abdel-Rahem
Prof. Sebsebe Demissew, Examiner	Sebl Demissew
Dr Kifle Dagne, Advisor	Kifle
Dr Sileshi Nemomissa, Advisor	
Dr Kifle Dagne, Chairman	Kifle

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Abstract

Chromosome data have contributed little towards resolving the problems of classification and phylogeny of the *Swertia* species (Yuan, 1993). For many species of the genus the chromosome number as well as morphology were not studied (Yuan and Küpfer, 1993).

In the present work, chromosome numbers were documented for six species of *Swertia* L. (Gentianaceae) from Ethiopia. *S. fimbriata*, *S. kilimandscharica*, *S. macrosepala* subsp. *microsperma* and *S. volkensii* var. *baleensis* were found to be diploid with 2n=26. *S. crassiuscula* subsp. *robusta* was diploid with 2n=20. The above chromosome numbers were determined from mitotic chromosomes. Diploid (2n=20, n=10) and tetraploid (2n=40, n=20) chromosome numbers were found for *S. abyssinica* from meiotic chromosome study. The chromosome numbers of *S. crassiuscula* subsp. *robusta*, *S. macrosepala* subsp. *microsperma*, *S. volkensii* var. *baleensis* and 2n=40 cytotype of *S. abyssinica* were reported for the first time. Further, the present study confirmed previous reports on the chromosome numbers of the remaining species and the basic chromosome number x=13 and x=10 for the genus.

Karyotypes of five species of *Swertia* namely, *S. fimbriata*, *S. kilimandscharica*, *S. macrosepala* subsp. *microsperma*, *S. crassiuscula* subsp. *robusta* and *S. volkensii* var. *baleensis* have been described and their similarities and differences were discussed. Even though the chromosome morphology of these species is very similar, some

variations in basic number, ploidy level, satellite number and size of chromosomes were observed among the *Swertia* species studied. The study revealed that the species were heterogeneous. It is recommended that further study of karyotype of all the species of the genus including chromosome banding is necessary to complete the cytotaxonomic knowledge of the genus as a whole.

I.INTRODUCTION

The genus *Swertia* belongs to the family Gentianaceae. It is a highly diversified and widely distributed genus. It comprises of about 150 species. In Africa, the genus comprises of 30 taxa which are distributed between 14^0 N and 30^0 S latitude in sub-Saharan countries, extending from West Africa to Madagascar (Sileshi Nemomissa, 1997; 1998). Sileshi Nemomissa (1994) identified three geographical distribution patterns for the African taxa of the genus and he critically considered them along with phenetic character states of the group as a whole. These geographical distribution patterns are: a) species which are widely distributed without any sign of infraspecific speciation and showing continuous variations in the taxonomically useful character states (e.g., *S. fimbriata, S. kilimandscharica* and *S. quartiniana*); b) species which are common to Ethiopia and Tropical East Africa and exhibit infraspecific segregation (e.g., *S. crassiuscula* s.l.); and C) endemic species (e.g., *S. macrosepala* subsp. *microsperma* and *S. volkensii* var. *baleensis*).

With regard to the study of the genus, morphological and pollination studies were carried out on some Himalayan species of *Swertia.* Some of these species were also studied cytolgically (Khoshoo and Tandon, 1963). In the African *Swertia,* taxonomic character states (Sileshi Nemomissa, 1994, 1997) and pollen morphology (Sileshi Nemomissa, 1996) were studied and used in the systematics of the genus.

There is no doubt that cytological characters, especially chromosome numbers and karyotypes play an important role in modern plant taxonomy (Yuan, 1993). Interspecific chromosomal diversification (variation) allows several systematically relevant conclusions

(Wien and Linz, 1978). Anderson (1937) suggested that taxonomy and cytology study the same phenomena.

Some species of the genus *Swertia,* along with their relatives were cytologically studied by Rork (1949). She published her work on a large number of species of the family Gentianaceae with the hope of improving the infrafamilial classification using cytological data. However, the study involved only the determination of chromosome number, not karyotypes. Inspite of the attempts made by some workers, [e.g., Rork (1949), Love (1953), Thulin (1970), Hedberg and Hedberg (1977) and Sileshi Nemomissa (1994)] to study the chromosome numbers of *S. abyssinica*, *S. engleri* var. *engleri*, *S. lugardae*, *S. tetrandra*, *S. fimbriata* and *S. kilimandscharica*, the cytological work so far done on the genus is rather scanty. According to Yuan and Küpfer (1993), among 150 species of *Swertia*, only 41 species have been investigated cytologically.

Smith (1970) emphasized the importance of a phylogenetic system of classification in which all the various categories are classified according to their evolutionary relationships. Khoshoo and Tandon (1963) remarked that a precise evolutionary picture of the genus *Swertia* will emerge only after detailed morphological, cytogenetical and ecogeographical studies are made.

Swertia is a genus with large number of species. The cytology of the genus is not well known. So far, only limited cytological information of taxonomic importance have been documented. Thus, the present study was undertaken with the following objectives.

General objective

- To study chromosome morphology of some species of the genus *Swertia* from Ethiopia.

Specific objectives

- To document the chromosome numbers where chromosome numbers were not reported.
- To confirm the chromosome numbers where chromosome numbers were reported.
- To describe the karyotypes of the species under study.
- To see phylogenetic relationships among the species studied using the cytotaxonomical data.

II. LITERATURE REVIEW.

A. Brief Description of the Genus *Swertia*

1.Systematic treatment

The species concept used in the systematics of the genus *Swertia* is mainly based on the discontinuity of morphological character states. In some cases, however, the concept is used for distinction of sympatric species. For some species that grow together in their natural habitat (sympatric species) the absence of intermediates was used as a criterion to indicate effective reproductive isolation (Sileshi Nemomissa, 1998). Sileshi Nemomissa (1994) used this species concept for taxa that are geographically separated on a large scale and have a distinct discontinuous variation in at least one morphological character.

According to Sileshi Nemomissa (1994), varietal rank is approached in two ways: 1) Geographically separated populations of a species which display only partial differences in one morphological character were treated as varieties. 2) Varietal rank is also given where an assemblage of specimens displays different statistical values for combination of characters as to form a complete discontinuity. In this case, the assemblage overlaps either altitudinally and/or ecologically.

2. Stems and growth form of *Swertia* species

The growth habit of the species of *Swertia* is either annual (e.g. *S. abyssinica* and *S. tetrandra*) or perennial (e.g. *S. engleri, S. fimbriata, S. kilimandscharica* and *S. crassiuscula*).

All species are characterized by their herbaceous and 4-angled stems. The stems are branched at the inflorescence region (e.g. *S. abyssinica)* or branched from their base (e.g., *S. lugardae*) (Sileshi Nemomissa, 1994). Further more, stems could be decumbent basally and abruptly erect towards the inflorescence region (e.g. *S. engleri*) or strictly erect (e.g. *S. kilmandscharica,* Fig. 7). At the point of their contact with the ground, the stem of the East African *S. crassiuscula* form new plantlets which is a means of vegetative propagation, a situation not encountered in any other *Swertia* species of North East Africa (Sileshi Nemomissa, 1994). Such a tendency towards a supplemental vegetative reproduction also characterizes some other tropical East African *Swertia* species, *S. uniflora* and *S. macrosepala* subsp. *macrosepala*. Vegetative propagation as a means of reproduction is developed only in some afroalpine *Swertia* species.

Sileshi Nemomissa (1994) pointed out that, the tallest African *Swertia* species reaches a stem height of 1.5 m (e.g. *S. kilimandscharica*) and the shortest stem length is about 2 cm (e.g. *S. lugardae*). The afroalpine *Swertia* species, e. g. *S. crassiuscula*, *S. macrosepala* and *S. volkensii* have developed xeromorphic leaves which could be viewed as an adaptation to afroalpine climate. The climate of this region was characterized by Hedberg (1964) as "summer every day and winter every night." On high mountains of tropical and Northeast Africa the climate is very warm during the day and very cold (with frequent frost) during the night.

3. Phytochemical investigations of *Swertia* and other Gentianaceae

Phytochemical study was carried out on some members of the family Gentianaceae, including *S. kilimadscharica* by Schilling (1974). He detected the presence of the

compound L-(+)-Bornestol in the family. Ghosal *et al.* (1973a) reported, for the first time, the presence of 1,3,5,6- tetraoxygenated xanthones in *Canscora decussata* of the family Gentianaceae. Xanthones were common in *Swertia* species and are also found in other genera of Gentianaceae, *Gentiana* and *Halenia* (Stout *et al.*, 1969; Stout and Fries, 1970), which indicates a close phytochemical relationship between these genera and *Swertia*.

The occurrence of mangiferin, both in the roots and aerial parts, and the presence of only one xanthone (1,8-dihydroxy-3, 5-dimethoxyxanthone) were reported by Ghosal *et al.* (1973b) for *S. chirata.* Ghosal *et al.* (1975) studied the xanthones of *S. bimaculata* and reported, for the first time, three new tetra-and penta-oxygenated xanthones and xanthone-o-glucosides in the genus.

In general, xanthones oxygenated at 1,3,5,8 positions and 1,3,7,8 tetraoxygenation patterns (as the hydroxylated and methoxylated derivatives) are common in *Swertia* species. Such oxygenation patterns are also common in *Gentiana*. This indicates that *Swertia* and *Gentiana* are related from the phytochemical point of view (Ghosal *et al.*, 1975). Generally, xanthones were employed for chemotaxonomic purpose by their chemical and spectral properties (Carpenter *et al.*, 1969; Chaudhuri and Ghosal, 1971).

A recent study, aimed at isolation and bioassay of the tropical East Africa species, *S. brownii,* has reported a strong anti-fungal activity of xanthones of this species against *Cladosporium cucumerinum* and *Candida albicans* (Rodriguez and Wolfender, 1995). A new flavonone-xanthone glucoside isolated from the Chinese *S. franchetiana* was found to be potent inhibitors of the DNA polymerase activity of human immunodeficiency virus-1 reverse transcriptase (HIV-1 RT) (Pengsuparp *et al.*, 1995). This biologically active

compound was found to be not cytotoxic with cultured mammalian cells. The authors have also elucidate how this active compound brings about the inhibition of the activity of HIV-1 RT. They found that Swerti francheside (isolated from *S. franchetiana*) binds to DNA and was shown to be a competitive inhibitor. Most reverse transcriptase inhibitors can contribute to *in vivo* toxicity and hence the discovery of agents capable of specifically inhibiting HIV-1 RT without mediating a toxic response remain of high priority. Natural products serve as one source of structurally novel chemicals that are worth investigating as specific inhibitors of HIV-1 RT.

4. Medicinal importance of *Swertia* species

Some species of the genus are known to have medicinal importance. For example, *S. chirata* is an important medicinal species (Wawrosch *et al.*, 1999). It is used very extensively in the indigenous system of medicine for a number of diseases and in particular it is used as a bitter tonic and laxative in South India. *S. chirata* is included in pharmacopoeias of Britain and the United States as well (Khoshoo and Tandon, 1963).

5. Geographic distribution of *Swertia*

5.1. General distribution of *Swertia*

The genus *Swertia* occurs mainly in temperate regions and has the highest species concentrations in Himalya and Southwestern China (Sileshi Nemomisa, 1998). In Africa, *Swertia* species occur in sub-Saharan countries and Madagascar between 14^0 N and 30^0 S latitude (Sileshi Nemomissa, 1994).

5.2. Distribution of *Swertia* in Ethiopia

Swertia is distributed in the afromontane and afroalpine regional center of endemisim (White, 1983). With regard to the flora area of *Swertia*, Sileshi Nemomissa (1994) considered that "North East Africa" is synonymous with Ethiopia and Eriteria. The reason is that *Swertia* occurs in no other country in that region other than these countries. The distributions of some *Swertia* species in Ethiopia are shown in Fig. 1-3. Regional floristic divisions of Ethiopia used for the description is that adopted from Hedberg and Edwards (1989).

According to Sileshi Nemomissa (1994), *S. lugardae* mainly inhabits Simen (Gondar) and Bale Mountains. It also occurs in Arsi (e.g. Mt. Chilallo, Mt. Kaka), Ankober and Debre Sina, Gughe highlands (Gamo Gofa). *S. usambarensis* is mainly distributed on the highlands of Sidamo and Gamo Gofa; *S. quartiniana* in Keffa. On the other hand, *S. engleri* s.l. is recorded from Mt. Zuqala and Mt. Kundi (Shewa), Hararge and Mt. Guna (Gonder). It is also found in Wello (montane area) at the turn of Lalibela from Woldiya to Woreta road.

In NorthEast Africa, *S. crassiuscula* subsp. *robusta* is recorded only from Bale Mts., Boro Lucu, Galama and Mt. Chilallo (Fig. 1). Both *S. macrosepala* subsp. *microsperma* and *S. volkensii* var. *baleensis* are endemic to Bale Mountains. *S. fimbriata* and *S. kilimandscharica* (Fig. 2-3) are distributed on Mt. Chilallo, Mt. Kaka, Bale and Simen Mountains (Sileshi Nemomissa, 1994).

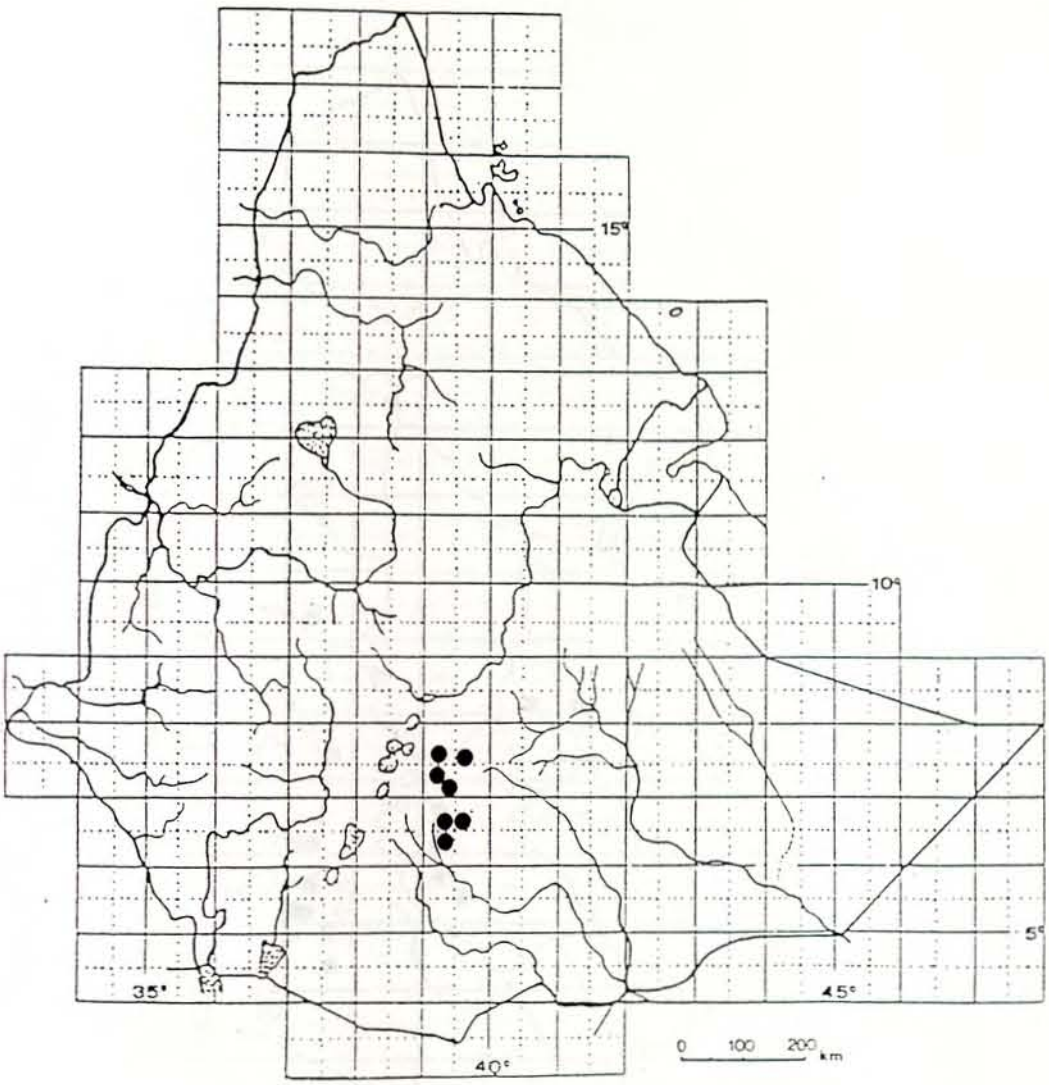

Fig. 1. Distribution of *Swertia crassiuscula* subsp. *robusta* in Ethiopia (Sileshi Nemomissa, 1994).

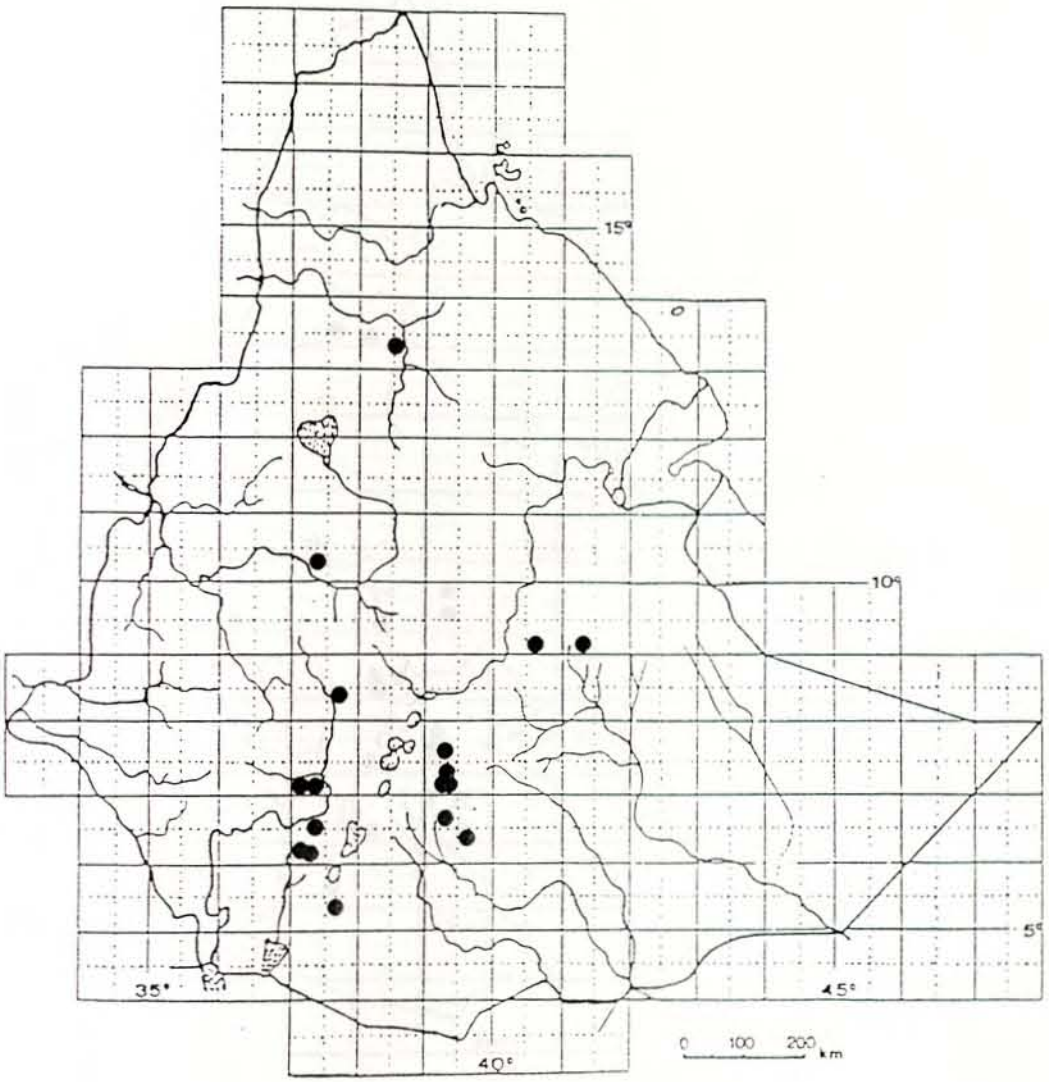

Fig. 2. Distribution of *Swertia kilimandscharica* in Ethiopia (Sileshi Nemomissa, 1994).

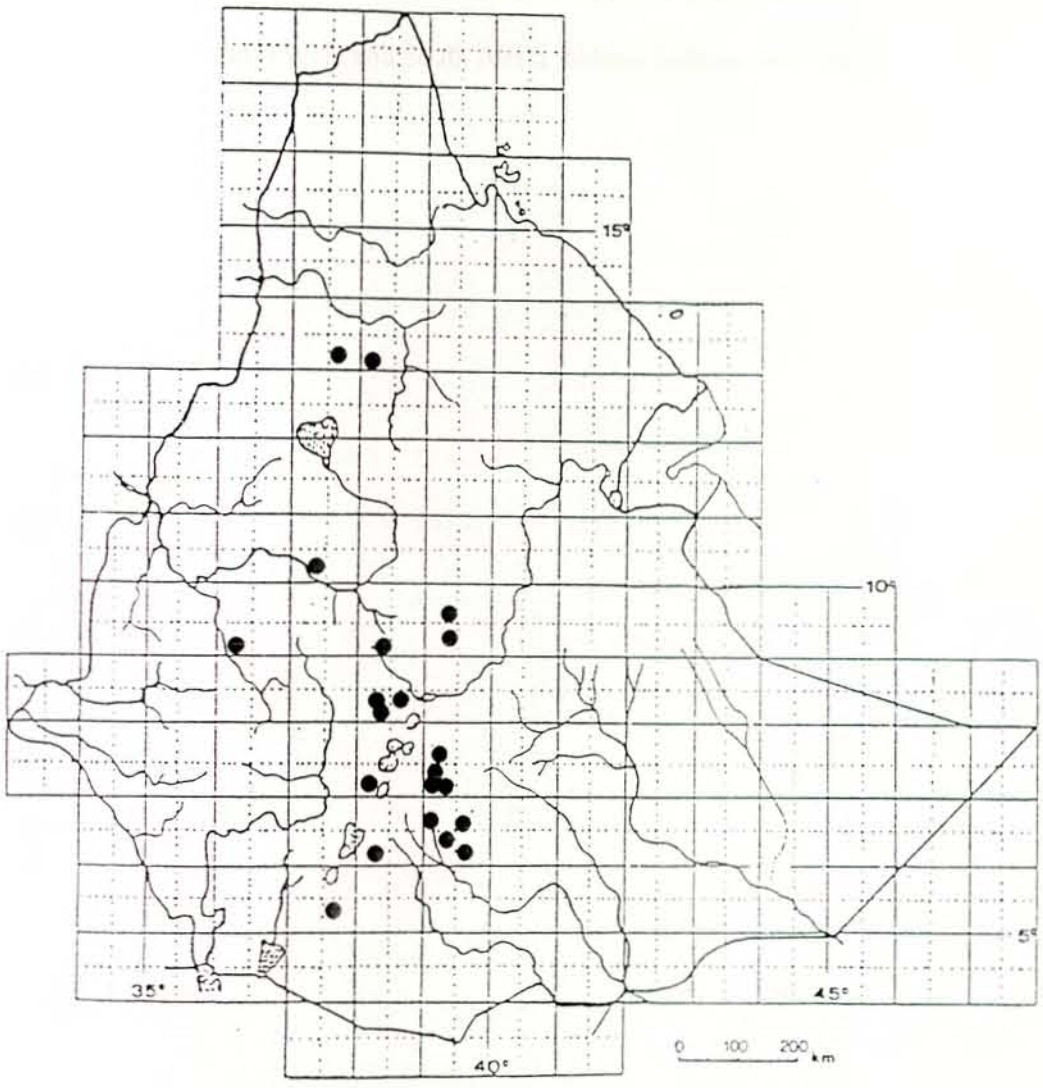

Fig.3. Distribution of *Swertia fimbriata* in Ethiopia (Sileshi Nemomissa, 1994).

4.3. Endemism

S. pumila, *S. crassiuscula* subsp. *robusta*, *S. scottii* and *S. engleri* were found to be endemic to Simen and Bale mountains. The remaining *Swertia* species in this region either occur on the mountains of Tropical East Africa or elsewhere outside this region in Africa e.g. *S.welwitschii* occurs in Angola and South Africa (Sileshi Nemomissa, 1994).

B. Karyotype and Its Systematic Significance

The karyotype is defined as the phenotypic appearance of the somatic chromosomes in contrast to their genic content (Jackson, 1971; Fristrom and Spieth, 1980). The major components of karyotypes which can be observed for comparison of related species include (1) absolute chromosome size; (2) chromosome morphology; (3) relative chromosome size and (4) variation in chromosome number such as aneuploidy and polyploidy (Sharma, 1991).

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 similar chromosomes than those of more distantly related ones (Sharma, 1991). So the study of chromosome morphology is a powerful instrument in the resolution of taxonomic difficulties and in the tracing of evolutionary relationships (Smith, 1970).

Chromosome morphology is studied from the somatic chromosomes (Jackson, 1971; Fristrom and Spieth, 1980). In order to study the detailed morphology, and then prepare a good karyotype, suitable fixation is an absolute necessity (Newcomer, 1953). Sharma (1956) suggested that critical fixation signifies clarification of chromosome morphology to minute details, in which both primary and secondary constriction regions come out extremely pronounced.

Carnoy fixation (3:1, alcohol:acetic acid) is the most widely used technique in the preparation of chromosomes for microscopic examination. For example, Yuan (1993), Yuan and Küpfer (1993) and Kifle Dagne (1994b) used this fixative to fix chromosomes.

After similar fixation techniques and exposure to the same stains at the same concentrations, the chromosomes of different species vary greatly in their ability to absorb the dyes on their surfaces (Stebbins, 1971). In addition, the chromosomes of many species contain some regions, which stain more easily than others at many stages of the mitotic cycle. These have been designated as heterochromatic regions (dark staining regions) of the chromosome (Brown and Nur, 1964; Brown, 1966; Weaver and Hedrick, 1992).

1. Components of the karyotype

1.1. Chromosome number

Chromosome number remains constant within species, which make it useful information for taxonomic purposes. The normal 2n chromosome number is worked out from the frequency of nuclei with the same complements (Sharma and Talukdar, 1957; Sharma and Sharma, 1959). Related species may have the same or different chromosome numbers. Variation of chromosome numbers may occur among individuals of a species as a result of aneuploidy or polyploidy (Sharma, 1991).

a. Basic chromosome number

Basic chromosome number (x=n) is defined as the number of chromosomes in a set (Snustad *et al.*, 1997). Basic number is one of the most widely used characters in biosystematic studies and there have been a vast amount of phylogenetic speculations using this value as a guide (Jones, 1970). Each species has its own specific basic number.

Reciprocal translocations, with at least one of the breakage points being in inert (hetrochromatin) regions, can transfer euchromatin to a centromere carrying only inert chromatin to give an added chromosome, or can transfer all of the euchromatin from one chromosome to another, with loss of the depleted and now inert chromosome, to reduce the basic number (Swanson, 1965).

b. Polyploidy

Polyploidy is the case where chromosome complements are consisting of three or more basic sets (Mettler and Gregg, 1969; De Wet, 1971; Grant, 1971). It is numerical differences with respect to whole sets of chromosomes (Grant, 1971; Snustad *et al.*, 1997). This definition applies to whole organisms, organs, tissues or single cells (Jackson, 1976). Polyploids arise from chromosome doubling in somatic cells or through the functioning of cytologically unreduced gametes (Rhoades and Dempsey, 1966; Snustad *et al.*, 1997). Polyploid cells are often encountered among diploid cells in vegetative tissues of most plants. These polyploid cells occasionally lead to the development of polyploid branches, the production of diploid gametes, and eventually polyploid individuals (De Wet, 1971). Such type of polyploidy is referred as autopolyploidy in which chromosomes at meiosis are associated into univalents, trivalents and quadrivalents instead of the usual bivalent association.

De Wet (1971) has stressed that most polyploids probably originated as a result of hybridization between either well-differentiated ecotypes within a species or between closely related species which is followed by chromosome doubling. Such type of polyploidy is known as allopolyploidy where the normal bivalent association of meiotic chromosomes were observed. Polyploids generally have different ecological requirements

than their diploid parents (Johnson and Packer, 1965; Dubuc and McGinnis, 1970; De Wet, 1971).

Polyploidy and adaptation

The primary phylogenetic effect of polyploidy is to stabilize selected hybrid genotypes which are intermediate between or represent recombinations of the characteristics found in the ancestral diploids (Stebbins, 1966).

Polyploids that arise within an ecotype of species are usually assumed to have contributed very little towards evolution. No new genes are added to the population through polyploidy and mutations are successfully masked by it (Klekowski and Baker, 1966). So, mutations are far more effective in diploids than in polyploids (De Wet, 1971).

Polyploidy and speciation

Polyploidy played a significant role in the origin and evolution of many plant species (Avers, 1980). Beside stabilization of selected hybrid genotypes, polyploidy provides a mechanism by which daughter and parental populations become immediately isolated from each other genetically (De Wet, 1971). However, isolation is rarely complete. Particularly following polyploidy, some gene exchange usually takes place between populations of different ploidy levels. Even such a well-established polyploid as *Triticum durum* (tetraploid wheat) introgresses with its wild diploid relatives in the Near East (Vardi and Zohary, 1967).

Change in chromosome number and morphology is often associated with spèciation (Lewis, 1962; Doyle, 1963). Stebbins (1966) indicated that the availability of pioneer habitats, and Lewis (1966) pointed out that environmental extremes are directly or indirectly conducive to chromosomal organization. Changes in karyotype may have a selective advantage under these conditions, or they may become fixed by strong reduction in population size during climatic changes. White (1968) suggested that changes in chromosome number and morphology may contribute substantially towards speciation in plants, insects and probably all other groups of animals. Evidence in the case of most polyploid series is that the lower numbers are primitive and the higher ones are derived (Stebbins, 1971).

c. Aneuploidy

Aneuploidy involves an increase or decrease in chromosome number by less than a complete set (Jackson, 1971). It refers to differences in number of individual chromosomes (Grant, 1971).

Snustad *et al.* (1997) explained the distinction between aneuploidy and polyploidy as follows. Aneuploidy refers to numerical change in part of the genome, usually just a single chromosome, whereas polyploidy refers to a numerical change in a whole set of chromosomes. Aneuploidy implies a genetic imbalance, but polyploidy does not.

d. B Chromosomes (Accessory chromosomes)

In addition to the normal constant complement of chromosomes, many species of plants and animals contain a variable number of chromosomes, which have much smaller size and

are usually heterochromatic (Fröst, 1969; Rhoades and Dempsey, 1972). These chromosomes are known as B chromosomes or supernumerary chromosomes (Peeters *et al.*, 1985; Snustad *et al.*, 1997). They are not part of the normal karyotype, since they vary in number between tissues, organs and cells of the same individual, between individuals within a population and between populations (Bicolor and Sannomiya, 1974).

Bs are generally thought to be devoid of genes, although in some instances they are known to affect fertility (Weaver and Hedrich, 1992). B chromosomes are commonly considered as parasitic (Nur, 1977) or selfish (Matthews and Jones, 1983) and employ premeiotic, meiotic or postmeiotic accumulation mechanisms to counteract selection pressure against them (Rees and Jones, 1967; Matthews and Jones, 1982). For example, Kifle Dagne (1994a, b) reported that the accumulation mechanism of Bs in *G. scabra* subsp. *scabra* is premeiotic.

1.2. Chromosome morphology

As indicated previously, chromosome morphology is studied from the somatic chromosomes (Jackson, 1971; Fristrom and Spieth, 1980). Meiotic chromosomes are not suitable for morphological investigation and they are not generally studied from this aspect (Sharma, 1956).

Chromosome morphology is usually studied at the metaphase of mitosis, when chromosomes become contracted to the maximum or nearly so and when they are most easily stained (Stebbins, 1971; Sharma, 1991; Snustad *et al.*, 1997). Chromosomes are arrested at mitotic metaphase especially after treatment with spindle disrupting or inhibiting

chemicals, such as colcemid, colchicine, 8-hydroxyquinoline, bromonapthalene, vinblastine or griseofulvin (Jackson, 1971). Cold treatment in ice water is also as effective as spindle inhibiting chemicals (Dermen, 1940).

Chromosome size and morphological landmarks such as centromeric positions, secondary constrictions and satellites are useful for chromosome identification and for the characterization of karyotypes.

1.2.1. The centromere

The centromere is identified as a large constriction where the chromosomes appear to be pinched (Weaver and Hedrick, 1992). In the morphologic identification of chromosomes, the location of the centromere is the most useful landmark (Levan *et al.*, 1964). This is due to constant size, shape and position of the centromere for each chromosome (Longley, 1941; Garber, 1972). In mitosis, the centromere can be observed best at late prophase or metaphase, especially after treatment with spindle inhibiting chemicals, such as colchicine or 8-hydroxyquinoline (Jackson, 1971). Based on the centromeric position in relation to the chromosome arm (region on either side of centromere), chromosomes could be described as follows:

Telocentric: centromere located at one end of the chromosome, so that the chromosome consists of single arm.

Acrocentric: centromere located near one end of the chromosome, so that it contains one long arm and one very short arm.

Sub-metacentric: centromere located near one end of the chromosome than the other, so that the two arms are distinctly unequal, but less so than in acrocentric situation.

Metacentric: centromere located at or near the middle of the chromosome, so that its arms are nearly or quite equal in length.

Some other authors have used the terms terminal, sub-terminal, sub-median and median for the corresponding telocentric, acrocentric, sub-metacentric and metacentric positions of centromeres (Longley, 1941). There were also cases where the same terms were used in different senses by different workers or by the same worker in different occasions (Levan *et al.*, 1964). So there has been confusion of terms for chromosomes with specific centromeric positions. To avoid this problem, Levan *et al.* (1964) have proposed a very useful chromosome nomenclature based on centromeric position. They recomonded the terms median point (M), median region (m), sub-median region (sm), sub-terminal region (st), terminal region (t) and terminal point (T). These correspond to arm ratios of 1.00, 1.00-1.70, 1.70-3.00, 3.00-7.00, 7.00-∞ and ∞, respectively.

The study of chromosome fragments showed that centromere is important for the continuity of chromosome. Due to the fact that the centromere is the site of spindle fiber attachment, and thus essential for chromosome movement, only chromosome fragments that include centromere can be carried on from one cell generation to the next, whereas chromosome fragments without centromere soon disappear (Longley, 1941; Jackson, 1971).

1.2.2. Secondary constriction and satellite

Some chromosomes in the complement posses a second constricted region other than the centromere. This region is known as secondary constriction. It is located between the centromere (primary constriction) and the end of chromosome arm (Garber, 1972; Weaver and Hedrick, 1992). The secondary constriction may be uncoiled or slightly coiled and of smaller diameter than adjacent areas at prophase or metaphase, or it may be the same diameter and similarly coiled as adjacent regions but less stainable (Stebbins, 1971). For higher plants and animals, the entire length of the secondary constriction may be synonymous with the nucleolar organizer (Jackson, 1971).

The part of the chromosome arm distal to the secondary constriction is referred to as the satellite. Though species may differ in the number and size of satellites, a diploid species has at least a pair of satellited chromosomes. Differences in the number of satellites indicate differences in the number of nucleolar organizer regions. Differences in the size of satellites indicate the position of the nucleolar organizer regions relative to the end of the chromosome (Stebbins, 1971; Walker, 1971; Weaver and Hedrick, 1992).

Two closely related species may differ in the number and location of their nucleoli organizer regions and consequently in the number of satellited chromosomes and satellite size respectively. For example, Jackson (1971) reported differences in the number of nucleolar organizer regions between the two closely related *Chironomus* species. According to him, *C. tentans* has nucleolar organizer regions on two pairs of chromosomes, chromosome two and three, whereas there is only one pair of nucleolar organizer region in the diploid complement of *C. pallidivittatus*.

1.2.3. Relative chromosome size.

The chromosomal complements of most species of plants consist of chromosomes of comparable size. However, chromosome complements of many species contain chromosomes of two contrasting sizes, large and small. Such type of chromosome size variation is found, for example, in the genera of *Aloe* and *Gasteria* (Vosa and Bennett, 1990). In other instances, a graded series of chromosomal sizes exists, as it is true for many animals including man (Stebbins, 1971).

The relative chromosome size, as a component part of a karyotype is, therefore, used to characterize chromosomes. Longley (1941) and Hedberg (1970) suggested that it is necessary to obtain a large number of measurements of each chromosome so as to have dependable data to show the relative length of chromosomes. The reason for this is that, the condensation process may not take place always at the same rate in all chromosomes or even in different sections of the same chromosome, which indicates that information from a single cell cannot give reliable information about relative size of chromosomes.

1.2.4.Absolute chromosome size

Absolute chromosome size (including the total DNA content of the nucleus) may vary between genera of the same family having the same or similar basic chromosome numbers and even between species of the same genus (Stebbins, 1971; Sharma, 1991). Stebbins (1971) indicates that, in different groups of flowering plants, trends towards

increasing chromosome size as well as toward decreasing chromosome size can be detected.

2.Karyotype evolution

Karyotype of an organism is not static. It evolves or changes through time. The ultimate goal for morphologic classification of chromosomes is to reach understanding of how this karyotypic evolution has proceeded and to find correlation with the evolution of other systematic characters such as anatomical, biochemical and behavioral (Levan *et al.*, 1964).

The evolutionary change of chromosomes involve, change in chromosome number, chromosome morphology, relative and absolute size of the chromosomes due to numerical or structural changes of chromosomes (Stebbins, 1971).

Chromosome numerical change in karyotype evolution may involve aneuploid or polyploid (autopolyploid or allopolyploid) change of chromosome number, which affects the number of chromosomes within the karyotype.

Karyotype evolution is also accomplished through the medium of chromosome structural changes which may occur within single chromosome or may affect two or more members of the karyotype simultaneously (White, 1973). Breakage of the chromosomes followed by recombination of the broken ends is often accompanied by gain or loss of parts. This leads to reconstituted karyotypes that may distinguish one species from another closely related form (Swanson, 1965; White, 1973). The number of chromosome breaks and the method of reunion determine the type of rearrangements (Grant, 1971). The major types of

chromosome structural changes include deletions (deficiencies), duplications, inversions (paracentric or pericentric) and translocations.

Duplications and deletions involve quantitative changes that result in gain or loss of chromatin material, respectively and these changes are known to affect the karyotype. Pericentric inversions (inversions which involves both arms of the chromosome), are instrumental in shifting the position of the centromere and can thus be responsible for variation in the karyotype (Swanson, 1965). Stebbins (1971) pointed out that differences in relative chromosome size could be brought about by segmental interchange involving translocations of unequal size.

Sometimes fusion or fission of chromosomes at centromeric region can change the karyotype, numerically as well as structurally without changing the total number of chromosome arms (fundamental number). For example, telocentric chromosomes can arise from metacentric or acrocentric chromosomes by means of breaks in the centromeric regions (Dermen, 1940; Stebbins, 1971). Conversly, metacentric chromosomes can arise by the fusion of two telocentrics (Jones, 1970). These changes are apparently very common in the evolution of animal species. In plants, however, telocentric chromosomes are rare (Stebbins, 1971).

Karyotype symmetry / asymmetry is a good expression for the general morphology of karyotype in plants (Matern and Simak, 1968; Romero, 1986). A symmetrical karyotype is one in which the chromosomes are all of approximately the same size and have median or submedian centromeres. Increasing asymmetry can occur either through the shift of centromer position from median to sub-terminal or terminal. It could also occur through the

accumulation of differences in relative size between the chromosomes of the complement, thus making the karyotype more heterogeneous. The general trend in the evolution of karyotype is known to be from symmetrical to asymmetrical (Stebbins, 1971). Sharma (1991) suggested that two trends can be observed in the progressive evolution from symmetrical karyotype to asymmetrical one: (1) Reduction in length of one arm of the chromosome giving rise to submedian and subterminal centromers from median ones; and (2) Reduction in size of some chromosomes in relation to others of the same set, so that the advanced karyotype has chromosomes of progressively unequal sizes.

The significance of karyotype asymmetry as indicative of the direction of karyotype evolution can be understood by looking for the association between increasing asymmetry and specialized morphological or ecological characteristics. For example, annual vs. perennial growth habit and the presence vs. absence of specialized morphological features.

Stebbins (1971) proposed a general trend of increasing asymmetry with increasing specialization. Nevertheless, Jones (1970) pointed out that the true course of events could only be understood by studying a group of closely related taxa.

3.Karyotype as an aid to taxonomic classification

Chromosome studies have proven themselves time and again to be powerful instruments in the resolution of taxonomic difficulties and in the tracing of evolutionary relationships (Smith, 1970). For example, Goodspeed and Thompson (1959) mentioned that the study of chromosome morphology of the genus *Nicotiana* contributes more evidence towards its taxonomic picture. In cytotaxonomic and phylogenetic studies of species, different

characterstics of karyotypes are usually observed and compared. Sharma (1991) emphasized that in comparing the karyotypes of different species six different criteria are usually taken into account, namely (1) variation in absolute chromosome size; (2) variation in the position of the centromere; (3) variation in relative chromosome size; (4) variation in basic number; (5) variation in number and position of satellite; and (6) variation in the degree and distribution of heterochromatic regions and repeated DNA segments.

Stebbins (1971) summarized chromosomal differences as taxonomic characters in the following way. Chromosomal differences in size, shape and number from one species to another species have meaning entirely different from morphological, physiological and biochemical differences of the species. These latter differences indicate variation in the products of gene action as modified by environmental factors during development. Therefore, their causal significance in evolution is related only to the adaptive value, which they possess.

On the other hand, chromosomal differences reflect more or less directly the genic content of the individual (Rees and Jones, 1967; Sharma, 1991). Differences in chromosome size may reflect differences in the number of different kinds of gene products or proteins produced by the individual (Barber, 1970; Stebbins, 1971; Sharma, 1991). It may also reflect the duplication of genes, which influence the rate at which a particular kind of protein can be synthesized. Chromosome morphology differences reflect variation in gene arrangement which can affect the way in which genes can become segregated and recombined. Finally, chromosome number differences may reflect either difference in gene arrangement or gene duplication or both (Stebbins, 1971; Sharma, 1991). So, Stebbins

(1971) concludes that in order to understand evolution, one must become familiar with and take into accounts all of these differences.

III. MATERIALS AND METHODS

A.Materials

Out of sixteen species of *Swertia* found in Ethipoia, 19 populations belonging to seven species were used in this investigation. The species names and populations along with their localities of origin and altitude are listed in Table 1. The materials were collected from field and grown in green house as indicated in Fig.5-8 for *S. crassiuscula* subsp. *robusta, S. macrosepala* subsp. *microsperma* and *S. Kilimandscharica.* The plant materials were collected from Addis Ababa, Mt. Chilallo, Bale Mts. National Park and Semen Mts. National Park.

Table 1. Collection sites of *Swertia* materials used in the present study.

Taxon	Locality	Altitude
S. abyssinica Hochst.	Michael Birru Sefer (A.A)	2600m.
	Dinsho (BMNP)	2800m.
	Garamba Dima (Bale)	2890m.
	Guracha River (BMNP)	2800m.
S. crassiuscula Gilg subsp. *robusta* Sileshi	Senatii (BMNP)	3800m.
	Hora Batu (BMNP)	3900m.
	Garba Guracha (BMNP)	3900m.
S. fimbriata (Hochst.) Cufod.	Guracha River (BMNP)	2800m.
	SMNP	3400m.
	Mt. Chilallo (Arsi)	2700m
S. Kilimandscharica Engl.	Fincha Habera (BMNP)	3400m.
	Garamba Dima (Bale)	2890m.
	On the way to Simbro (BMNP)	2850m.
	Senatti (BMNP)	3800m.
S. lugardae Bullock	SMNP	3400m.
	Senatti (BMNP)	3800m.
	Guracha River (BMNP)	2800m.
S. macrosepala Gilg subsp. *microsperma* Sileshi	Senatii (BMNP)	3800m.
S. volkensii Gilg var. *baleensis* Sileshi	Senatii (BMNP)	3800m

BMNP = Bale Mts. National Park.
SMNP = Semen Mts. National Park.
A.A = Addis Ababa

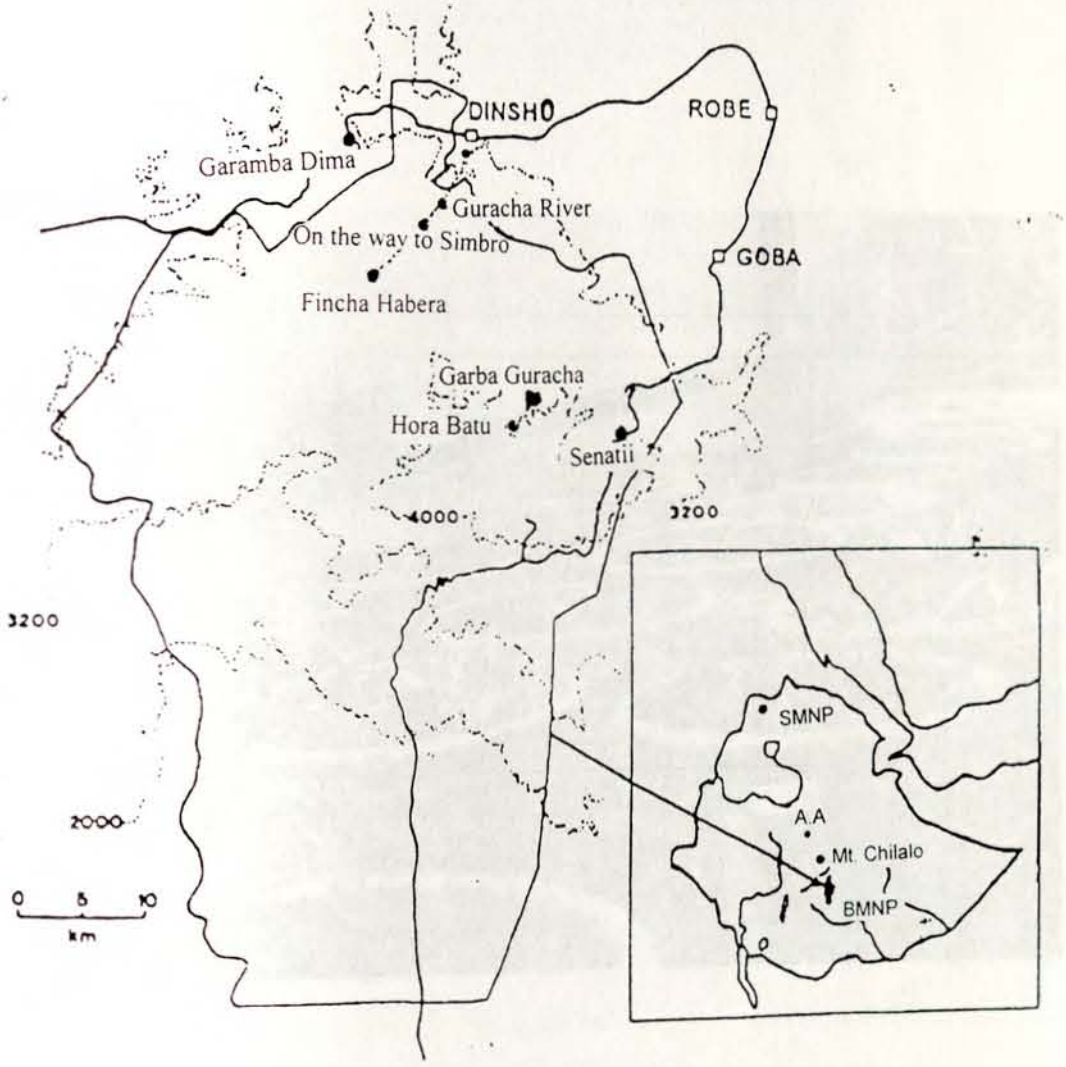

Fig.4. A map showing the sampling sites of populations of *Swertia* species. See text for further explanations.

Fig. 5. Some *Swertia* species collected from field and grown in green house (*S. crassiuscula* subsp. *robusta* and *S. macrosepala* subsp. *microsperma*).

Fig. 6. *Swertia crassiuscula* subsp. *robusta* grown in the green house.

Fig .7. *Swertia kilimandscharica* grown in the green house.

Fig. 8. *Swertia macrosepala* subsp. *microsperma* grown in the green house.

B. Methods

Somatic chromosome preparation

Pretreatment and maceration: Live plants were collected from field (Fig.4) and grown in pots in green house (Fig.5-8), and were used as source of root tips for chromosome preparations. Actively growing roots were collected into vials from well-established potted plants. The roots were cold treated by placing the vials containing roots in a Petri dish and covering the Petri dish with ice water for 24 hours. The cold treated roots were fixed in ethanol-acetic acid (3:1) for about 1h. The fixed roots were either used immediately or stored in 70% alcohol at 4°C until used. The roots were rinsed in distilled water, and their tips were excised on glass slide. The root tips were then treated with drops of 3% cellulase-pectinase solution at 35 °C - 37 °C for about 1h (Kifle Dagne and Heneen, 1992). Then the enzyme solution was carefully blotted off using filter paper and the root tips were rinsed with drops of distilled water. The root tips were then used to make squash or air-dry preparations as follows:

Aceto-orcein squashing: After enzyme-macerated root tips were rinsed in distilled water, the water was blotted off using filter paper. The root tips were mashed in a drop of aceto-orcein, covered with a cover slip and squashed under a filter paper. Preparations were made semi-permanent by sealing the edge of the cover slip with a mounting medium.

Air-dry preparation: Air-dry preparations were made by mashing the enzyme-macerated root tips in a drop of ethanol: acetic acid (3:1) on a glass slide. The slides were allowed to

air dry under room temperature. When staining was desired, a drop of aceto-orcein stain was placed on an air-dry preparation, covered with a cover slip and after a few minutes of staining, the excess stain was removed by pressing on the cover slip with filter paper. The edge of the cover slip was sealed to prevent the preparation from drying.

Photography: Chromosome photomicrographs were taken from well spread chromosome preparations of semi-permanent slides.

Determination of chromosome numbers and karyotype: Chromosome numbers were counted from well-spread pro-metaphase and metaphase chromosome complements. The chromosome number for each population was determined from at least 60 different cells. Chromosome karyotype analyses were made from printed photographs. The well spread metaphase chromosomes were measured for arm ratio determination. The nomenclature (terminologies) used in defining centromeric positions such as m, median; sm, sub-median and st, sub-terminal were that recommended by Levan *et al.* (1964). Arm ratio measurements were made on enlarged photomicrographs.

Meiotic chromosome preparation

Aceto-carmine staining: For meiotic chromosome study, young flower buds were fixed in the field in ethanol-chloroform-acetic acid (6: 3: 1) for 24 h and stored in 70% alcohol at 4^0C until used. Buds were stained in Snow's carmine (Snow, 1963) for several days at room temperature. Pollen mother cells (PMCs) were released in a drop of 45% acetic acid on a glass slide and squashed under a cover slip. Slides were made semi-permanent by sealing around the edges of the cover slip with mounting medium. Chromosome photomicrographs were taken from chromosome preparations of the semi-permanent slides.

Determination of chromosome numbers: Chromosome number was determined from pollen mother cells at diakinesis, metaphase-I and anaphase-I stages.

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DECLARATION

The thesis is my original work, has not been presented for a degree in any other university and that sources of material used for this thesis have been duly acknowledged.

Name: Mulugeta Kebede Tesissa

Signature: _____

Date: June, 2000

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

Name	Signature
1. Dr. Kifle Dagne	_____
2. Dr. Sileshi Nemomissa	_____