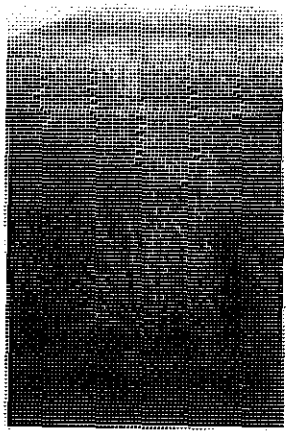

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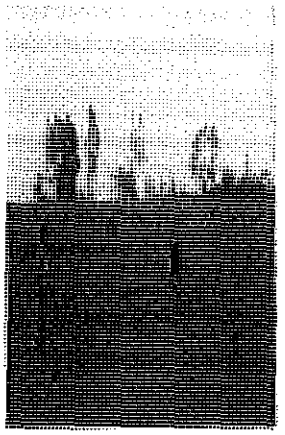

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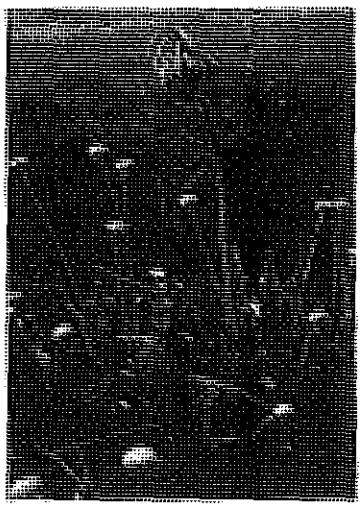
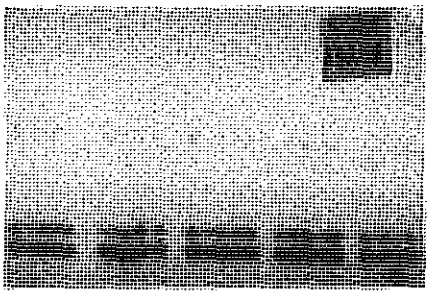


Tilahun Teklehaymanot

A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfilment of the requirements for the Degree of Ph.D. in the field of Genetics under the Biology Department, Faculty of Science



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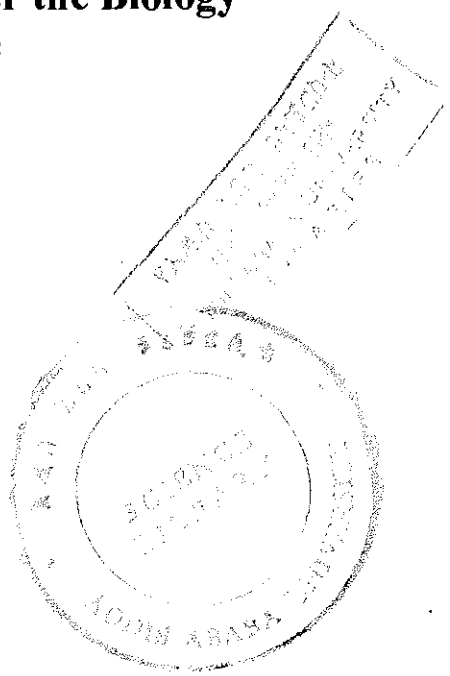

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Addis Ababa, 2001

DEDICATION

I dedicated this study to my late parents

W/ro MEAZA GENET SEBSEBE

&

ATO TEKLEHAYMANOT HABTEMARIAM

MAY GOD BLESS THEIR SOULS

ACKNOWLEDGEMENTS

This study was initiated by my advisors: Prof. Endashaw Bekele, Prof. Sebsebe Demissew and Prof. Inger Nordal to whom I would like to express my deepest gratitude and many thanks for their unreserved and extended consultations, guidance and limitless patience in proof reading. I would also like to thank Prof Morten M. Laane for his advice and kind permission to use the cytology laboratory.

I am also grateful to the staff members of the National Herbarium of Addis Ababa University (ETH) for their help throughout my study period, the guards at the experimental sites, data enumerators, and all my friends for their encouragement and support. My thanks are also extended to Mette Ursin, Thomas Marcussen, and the staff of the greenhouse of the University of Oslo for their kind assistance.

I would like to thank my family and my best thanks goes to my Wife W/ro Elifinesh Retta who shouldered the responsibility of the family when I was on the study leave, and my children: Biruk Tilahun, Abel Tilahun and Eskedar Tilahun.

At last but not least, I am highly indebted to Ambo College of Agriculture for the kind permission of the study leave and financial assistance, NUFU through the Petaloid Monocots Project for the overall financial assistance, Addis Ababa University and the University of Oslo for the scholarship grant and the Biology Departments of the respected Universities for allowing me to use their office, laboratory and library facilities.

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ABSTRACT

The study was conducted to work out the relation and conservation strategy of the endemic *Kniphofia* species by comparing their distribution, demography, reproductive biology, and genetic diversity. The *Kniphofia* species studied include *Kniphofia foliosa*, *K. hildebrandtii*, *K. insignis*, *K. isoetifolia*, *K. schimperi* and *K. pumila*.

The experiments performed were divided into field, greenhouse and laboratory. The field data were collected from nine experimental sites each with a size of 20 m X 20 m plot. The data collected from field and greenhouse include demography, reproductive and quantitative morphological phenotypic characters. The laboratory studies consist of karyotype, isoenzyme analysis and sequencing of *trn*L-*trn*F intergenic spacer of the chloroplast DNA.

The results obtained from the distribution data showed that the *Kniphofia* species are spread in waterlogged grassland to openings in the montane forest on the mountainous part of the country ranging from 2000 m.a.s.l. to 4000 m.a.s.l. *Kniphofia foliosa*, *K. isoetifolia*, *K. schimperi* are relatively widespread than *K. insignis* and *K. hildebrandtii*.

The results from demography and reproductive biology have shown that *Kniphofia* species are clonal plants that reproduce asexually and sexually. The meristems from the rhizomes give rise to new ramets that later produce their own rhizome. The new rhizome remains attached with the mother rhizome (underground stem). *K. foliosa* and *K. isoetifolia* have runners that give rise to new ramets that have the potential to be physiologically independent clones. *K. foliosa* has the highest and *K. hildebrandtii* the smallest number of ramets in the experimental sites. The presently studied *Kniphofia* species have protandrous flowers and pollinated by Tecezze sunbirds (*Nectarina tacazze*). The fruit to flower ratio ranges from 0.17 to 0.7.

Kniphofia species have 2n =12 number of chromosomes and the karyotype is 1m + 3sm + 2sm. The homologous pair in each species has different total chromosome lengths except the short arms of chromosome V and VI ($p< 0.05$). The dendogram made based on the morphology of the mitotic chromosomes indicated close relationship among all except *K. foliosa*.

The genetic diversity of the *Kniphofia* species as a whole is higher than what is described for endemic species. The total genetic diversity at the polymorphic loci ranges from 0.123 *K. foliosa* to 0.396 in *K. schimperi*. The genetic variation harboured among the species is only 6.6%. They have high genetic identity and very low genetic distance than congeneric species. Despite their closeness, *K. insignis* showed divergence on the cladogram made based on the genetic similarity values.

The trnL (UAA)3'-trnF (GAA) intergenic spacer of chloroplast DNA of the *Kniphofia* species has very low diversification among the species. The variations realized from the alignment of the sequences were one length mutation (indel) and four substitutions (transversion). The phylogenetic study of the species needs molecular markers that can provide sufficient parsimony-informative characters.

The results of the studies indicated that the two geographically restricted species (*K. hildebrandtii* and *K. insignis*) might need due attention when compared to the rest of the endemic species due to the land use changes taking place at present. Other wise, none of the studied population in the studied *Kniphofia* species are endangered because of genetic erosion or inbreeding.

1 INTRODUCTION

1. 1 Family Asphodelaceae

The genus *Kniphofia* Moench belongs to the family Asphodelaceae. According to Smith and Van Wyk (1998), the family includes two subfamilies: Asphodeloideae and Alooideae. These two subfamilies contain 17 genera and ca. 780 species that are mostly herbaceous and woody with trunks reaching to several meters high. These are mainly distributed in arid and mesic regions of the temperate, subtropical and tropical zones of the Old World. The main centre of distribution of the family is in Southern Africa.

According to Dahlgren *et al.* (1985), the family Asphodelaceae includes mostly herbaceous and some woody members with trunks reaching to several meters high. The members have no secondary thickening except in the species of *Aloe* and *Kniphofia*. The roots are inflated, fusiform and some with multi-layered velamen. Leaves are arranged spirally, and are dorsiventral, thick, succulent, and the shape varying from linear or subulate to thickly conical or even elliptical. The stomata are anomocytic or tetracytic, rarely paracytic. The vessels occasionally have scalariform perforation plates. The parenchymatous, aloine cells are arranged as a cup at the phloem pole of most vascular bundles of the leaf of Alooideae and are lacking in subfamily Asphodeloideae and these cells secret a range of anthraquinones and other substances. The inflorescence is spicate, racemose or paniculate. The flowers are bisexual, trimerous and hypogynous. The tepals are free to fused with their colour varying from white or rose-coloured to bright red and/or yellow purple and greenish. The stamens are 6 in number arising from the base of the ovary. The filaments are free from each other with dorsifixed-epipeltate anthers. The pistil is syncarpous, 3-carpellate and 3-locular with two numerous ovules per locule. It has long style with a small stigma of dry or wet type.

Placentation is axial. The fruit is a loculicidal capsule and contains arillate seeds, which are elongate, ovoid and sometimes winged in some species of *Eremurus*. The endosperm cells stores lipids and aleurone. The embryo is straight, linear and occupies three quarter of the length of the endosperm.

Van Wyk *et al.* (1995) identified seven anthraquinones from the rhizomes of 46 species belonging to the subfamily Asphodeloideae in the genera *Asphodelus, Asphodeline, Bulbine, Bulbinella* and *Kniphofia.* One of which, 1,8-Dihydroxyanthraquinones based on chrysophanol unit was found to be the main constituents of the subterranean metabolism of subfamily Asphodeloideae. But the genera *Bulbine, Bulbinella* and *Kniphofia* showed kniphofoline-type compounds, which characterizes the three genera from the rest of the subfamily and they concluded that the genus *Kniphofia* is not related to the Alooideae. Additionally, Van-Staden and Drewes (1994), and Dagne and Steglich (1984) isolated anthraquinone knipholone from fresh bulbs of *Bulbine latifolia* and *Bulbine frutescens* that supports the placement of *Kniphofia* and *Bulbine* within the subfamily Asphodeloideae.

Beaumont *et al.* (1985) showed the difference between the genus *Kniphofia* and the other genera in *Aloe, Chamaealoe, Astroloba, Lamatophyllum, Gasteria, Haworthia, Asphodeline, Asphodelus, Bulbine, Eremurus* and *Trachyandra* based on the presence or absence of thin-walled parenchymatous cells in the inner bundle sheath of the species in the genera. The presence of a thin-walled secretory tissue together with the compounds secreted in many species under the genera suggested relationship among all except genus *Kniphofia*. In the genus *Kniphofia*, the inner bundle sheath has lignified sclerenchymatous cells that make it different from the other group.

of the Tropical Africa species. Baijnath (1980) worked on the leaf anatomy in order to assist in the classification of the genus.

The species of *Kniphofia* in general are herbaceous medium-sized perennial plants (Smith and Van Wyk, 1998) and show variation in size according to the site and water available (Marais, 1973). Their habitats range from low and wet Savannah grassland from about 900 m.a.s.l. to montane and alpine vegetation about 4400 m.a.s.l. *K. grantini* Bak. from Burundi (J. Levalle, Coll. No. 3072, at Kew Herbarium) represents an example of *Kniphofia* species from the Savannah vegetation and *K. thomsonii* Barker var *thomsonii* from Kenya (G. Aut., Coll. No. 92, at Kew Herbarium) represents the alpine habitat.

Sebsebe and Nordal (1997), recognised seven species of genus *Kniphofia* in the Ethiopian flora. The species concept followed was according to Sebsebe and Nordal (1997). They are *Kniphofia foliosa* Hochst., *K. hildebrandtii* Cufod., *K. insignis* Rendle, *K. isoetifolia* Hochst., *K. pumila* (Ait.) Kunth, *K. schimperi* Baker and *K. thomsonii* Baker. Of these, *K. foliosa, K. hildebrandtii, K. insignis, K. isoetifolia,* and *K. schimperi* are endemic to Ethiopia.

Kniphofia pumila and *K. thomsonii* are widely distributed from West Africa to Eastern and Central Africa. According to Marais (1973), *Kniphofia thomsonii* is common in Kenya, Uganda and Tanzania, in particular on Mount Killimanjaro.

Other studies conducted were on the secondary metabolites and *in-vitro* propagation. Berhanu *et al.* (1986) extracted aloe-emodin, aloe-emodin acetate, chrysophanol, islandicin and knipholone from the rhizomes, leaves, flowers, and fruits of *K. foliosa, K. insignis, K. isoetifolia, K. pumila,* and *K. schimperi.* They used anthraquinones to show the variation

among the species and as taxonomic markers in the Ethiopian *Kniphofia* species. The endemic *Kniphofia* species based on presence or absence of chrysophanol (anthraquinone) in the flowers were separated into two groups. *K. insignis* and *K .pumila* make one group and the rest of species the other. In addition to these, the study revealed that anthraquinones as the major secondary metabolites of the genus.

McAlister and Van-Staden (1996) indicated the applicability of *in-vitro* propagation in the genus *Kniphofia*. They propagated plantlets, which were successfully hardened off in a seedling mix in the mist house for 2 weeks. The plantlets were generated from the apical regions of a stolon of *Kniphofia pauciflora* on Murashige and Skoog medium supplemented with 100-mg l-1 myo-inositol, 3% sucrose and 0.8% agar. They used Kinetin (2 mgl) and NAA (1 mgl) to obtain shoot development. Furthermore, Nayak and Sen (1992) studied the behaviour of chromosomes and level of 4C nuclear DNA content of the root tips of regenerated plants derived from rhizome explant of *Kniphofia nelsonii* and *Kniphofia uvaria.* Their result indicated that there is no much difference between the plantlets. Lindsey *et al.* (1998) pointed out the importance of Kelpak, a seaweed concentrate prepared from *Ecklonia maxima* (Osbeck) Papenfuss to increase root growth and plantlet establishment when applied as a soil drench following the planting of in vitro grown plantlets of *Kniphofia pauciflora* Bak. Thus, plantlets regenerated *in-vitro* propagation can be a potential resources for conservation.

The genus *Kniphofia* is found useful in the field of Horticulture and is grown in home and botanical gardens. It is also used as cut flowers, which indicates its aesthetic and economical importance. The species of *Kniphofia* in cultivation are known by common names, such as red-hot pokers and torch-lights (Cox, 1986). Some naturalized garden escapes were reported

from Australia indicating their early uses as horticultural plants (Conran, 1987). Beside this, according to Dagne and Steglich (1984), Berhanu *et al* (1986), and Bringmann *et al.* (1999), the secondary metabolites from genus *Kniphofia* have medicinal uses. Such as, knipholone and related natural phenylanthraquinones are considered to be a new group of potential antimalarials and anthraquinone aloe-emodin known to exhibit antileukemic properties. Also, the roots of *Kniphofia foliosa* are used in traditional Ethiopian medicine for treatment of abdominal cramps. Furthermore, naturally occurring species of *Kniphofia* are important as honeybee-plants for pollen source and nectar (Fichtl and Adi, 1994).

In this study, the distribution, 'instant' demography, reproductive biology, variation in quantitative morphological Phenotypic characters, karyotype, genetic variation were considered and this is followed by their relationship studies based on chloroplast DNA sequence of the *Kniphofia* species in Ethiopia. Previously no comprehensive studies have been conducted to investigate their reproductive biology, demography, the genetic variation and the phylogenetic relationship of these species although the basic taxonomy has been studied as part of the Ethiopian Flora Project. This study may assist any future conservation plan, which tend to cover the habitats of the species of *Kniphofia* in Ethiopia.

2 LITERATURE REVIEW

The global loss of genetic diversity and species extinction, especially in area of high species diversity like in tropical rain forests, is taking place at an alarming rate. This could destabilize global climate and biochemical cycles thereby leading to potential disastrous effects. Therefore, it is necessary to conserve those areas with species of potential medicinal, agricultural, recreational and industrial values (Bekele, 1986; Falkner *et al.*, 1997; Ginsberg, 1999).

One of the serious threats to the maintenance of biodiversity is the fragmentation of habitats that occurs throughout the world and turned habitat into sets of isolated remnants. As a result, many natural plant populations have become small and isolated. These subdivided populations have a higher proportion of homozygotes as a result of genetic drift and Wahlund effect and thus may face risks of extinction because of loss of genetic variation or demographic factors (Saunders *et al.* 1991). Such as, endemic plants, which are in the lists of global conservation priorities that were once widespread, are now found in pockets of suitable habitats and their extinction might be a greatest and irreversible loss of genetic resources (Templeton *et al.*, 1991; Kelbessa *et al.*, 1992; Pieko-Mirkowa *et al.*, 1996; Ginsberg, 1999).

The present structure of endemic plant populations is based on the past history of their ancestors, abiotic and, biotic factors and on human activities, which causes their isolation, rarity and endangerment compared to their widespread relatives (Fréville, *et al.*, 1998; Bevill and Louda, 1999; Thompson, 1999). Their survival and fitness is dependent on performance, spatial arrangement, and genetic structure of the population, stochastic and catastrophic disturbances. These aspects are closely linked and are a continuum of reaction. Moreover, the

upsets in one aspect modify or determine survival and fitness of population and may eventually result in the extinction of the species (Holsinger and Gottlieb, 1991). The ability of endemic plants to persist depends on the interaction between their life history traits and the environmental factors (Fiedler, 1987; Ellstrand and Elam, 1993).

A species is defined as "rare" when it occurs in a small number throughout its range or has narrow geographical range. It suffers the risk of extinction if its present environment is changed even if it is not yet threatened with extinction (Rabinowitz, 1981). According to Reveal (1981), a species is considered as "endangered" if it is threatened by factors that reduce its capacity to reproduce and survive. Depauperate genotype, past or present climate changes, age of taxon, pollination biology, land use change, and the environment affecting the germination and growth of individuals could result to endangering species (Fiedler, 1987).

Most of the endemic plants and plants with clonal growth are identified as having low genetic diversity and being at a disadvantage in the future, although there is a need for complete documentation of the total distribution area that a species occupies. In addition, the combined dynamic factors related to their actual and potential existence are needed (Aparicio and Garicia-Martin, 1996; Witkowski and Liston, 1997; Bosch *et al.,* 1998). Furthermore, effect of loss of genetic diversity and other stochastic events, on the present or future survival of the species may be studied by comparing them with related plant taxa that are co-occurring and are likely to be exposed to similar stochastic and evolutionary pressure (Bevill and Louda, 1999).

2. 1 SPATIAL STRUCTURE OF POPULATION

Clonal plants, unlike the non-clonal plants, are capable of naturally producing potentially independent offspring by means of vegetative growth and have a capacity to multiply by both

sexual and vegetative reproduction (Harper, 1977). The genet (genetic unit), which grows from seed once established, branches to form new independent units; ramets (physiological) that remain connected to a parent for the time. This constitutes the basic characteristics of clonal growth (Hartnett and Bazzaz, 1983; Hutchings and de Kroon, 1994, Oborny, 1994).

Clonal plants include some of long-lived and invasive plants that are assumed more abundant in arctic, aquatic, shady habitats, and under nutrient-limited conditions. Clonal growth substantiates low population growth by means of vegetative reproduction and scarcely spatially distributed resources can be made available by division of labour (Callaghan *et al.*, 1992; Alpert, 1996). Like all other non-clonal plants, the spatial structure of clonal plant population is affected as the abiotic and biotic component of the environment varies throughout their habitat. These can affect the distribution and performance including plant weight, size, height, productivity and fitness of a population (Crawley and Weiner, 1991; Wijesinghe and Hutchings, 1997).

The spatial structure of the clonal plant depends on the relative magnitude of the two mode of reproduction; sexual or vegetative (Harada and Iwasa, 1996). The amount and spatial pattern of recruitment of sexually reproduced seedlings are influenced heavily by spatial distribution of flowers, dispersal mechanisms and seed establishment at the time of seed dispersal, which determines distribution of adult plants. Therefore, the spatial structure of population may be regular (uniform) or aggregate (clumped) than randomly distributed (Rabinowitz and Rapp, 1980). In some plant species seeds are dispersed in clumps and others are dropped close to mother plants in high density. Therefore, if all seeds are equally likely to germinate they give a clumped spatial structure of a population. But, in most cases, the spatial structure of the

population can be regular because seeds are dispersed farther away from the mother (Augspurger, 1983).

In addition to these, microhabitat has effect on the germination of seeds, which positively facilitate or inhibit germination of con-specific seeds. The clumping of seedlings may generate intra-specific competition and leads to density dependant mortality of seedlings. Moreover, the distribution of the seedling or spatial pattern of recruitment is affected by the spatial structure of established plant, competition with parent adult plants or predators attracted or pathogens harboured by the parent adult plants, which leads to scattered or clumped spatial population structure (Augspurger, 1983; Fisher *et al.*, 1991).

In clonal plants species, offspring produced vegetatively are found close to their parents. The degree of spatial clumping depends on the strategy of the genet in distribution of the ramets. It can be phalanx strategy where the ramets are compact or clumped together or guerrilla strategy where the ramets are connected with long internodes and wonders away from the genet (Harper, 1977). According to Schimid *et al.* (1995), the vegetative mode of reproduction in clonal plants is not associated to the size-fecundity relationships, and there is no evidence of a minimum size for clonal growth. The vegetatively produced ramets can remain connected to the parent plant for unlimited period or the connection can decay any time from the point that the ramet produced its own root (Hartnett and Bazzaz, 1983). The clonal plants have an advantage of nutrient and water reallocation between ramets within clonal fragments from more suitable microhabitat to less suitable, which may reduce the competition among the member of the same genet (Alpert, 1996).

The other features that could vary the structure of population are the plant weight, size, height, productivity and fitness. Productivity depends on spatial and temporal aspects of flowering, which influences the pattern of pollination, dispersal (Johnson, 1992; Murali and Sukumar, 1994) and the number of flowers, which mature to seeds. In most cases it is asymmetrical in population with different ages, which is composed of small number of large plants and many small ones.

The resource availability, competition and genetic differences have effect on the size hierarchy of a population (Liddle *et al.*, 1982; Cannell *et al.*, 1984). These factors affect the size of individual plants, survival and spatial structure of a population. The variation become more asymmetric as the difference in size increases among the plants. It has a major effect on lifetime reproductive success such that the frequency of flowering and mean annual fecundity is higher for plants from higher size classes than one from lower size classes. Because of the linear relationship between the minimum size to be attained and sexual reproduction output, thought size-fecundity relationships show significant genotypic variation and phenotypic plasticity.(Matthies, 1990; Schmid and Weiner, 1993). It also affects the productivity and persistence of the population, which ultimately measure the performance structure of a population in the future (De Jong and Klinkhamer, 1989; Kunin and Shamida, 1997).

2. 2 DEMOGRAPHY

Demographic studies helps to understand the birth and death rates or increase and decrease in the density of plants at particular life history stages, and have been used widely to model the dynamics of plant population (Harper, 1977; Silvertown *et al.*, 1993). The study reveals the population structure, genetic variability and vulnerability to natural selection. According to Silvertown and Lovett-Doust (1993), the relative change in number of organisms at a given

place per time in a population is describe by demographic parameters, where the finite rate increase or annual increase is described as $\lambda = N_t / N_{t-1}$ or the relative increase between the ratio of the demographic factors that contribute to change of dynamics of population; $N_t(B+I)/N_{t-1}(D+E)$. The net result determines whether the population remain stable, $N_t = N_{t-1}$; $\lambda = 1$, or increase $N_t > N_{t-1}$; $\lambda > 1$ or decrease $N_t < N_{t-1}$; $\lambda < 1$.

The prediction of the increase or decrease in population density does not tell anything about the mechanisms that controls the dynamics of population or growth of population that affects the demographic factors such as the age or stage in the life history of a population that is important to the annual net increase; λ. This may be identified by projection matrix or transition matrix: based on the age, stage, or combining both age and stage (Law, 1983; Silvertown *et al.*, 1993; Bullock *et al*, 1996; Vavrek *et al.*, 1997; Caswell, 1998; Rivas and Owens, 1999).

2. 3 GENETIC STRUCTURE

Genetic diversity is highly needed during adaptation to environmental changes and is necessary to preserve the long term-evolutionary potential of a species. It is responsible for the survival and reproduction of the organism in the contemporary and future environment (Clegg, 1997). It influences the physiology, demography, and performance of population or individual. It is the basic component of population and community, and for the evolution of higher level of biodiversity. It is an indispensable prerequisite for the function of our biosphere (Vida, 1994). It is found distributed or clumped in a plant population. The nonrandom distribution of genetic variation is often referred to as genetic structure of a population (Loveless and Hamrick, 1984).

The sum total of the genetic constitution (genotypes) of the member of a species makes the gene pool of the species, and the variation in the gene pool is expressed in terms of either genotypic or gene frequency (Brown *et al.*, 1990). The richness or evenness of alleles describes the degree and level of genetic diversity at gene level. Richness of allele measures the diversity and the total number of distinct allele at a locus in the population. Evenness measures frequency of the different types in the population or samples or lack of variation among the frequencies of alleles. The genetic diversity is high when the number of alleles is large and has equal or low variance in the frequency (Hamerick and Godt, 1989; Brown, 1990).

The genetic variation is measured by estimation of the proportion of polymorphic loci or polymorphism in a population and heterozygous individuals per locus or heterozygosity. The level and distribution is estimated based on morphological or phenotypic variations, electrophoresis of soluble proteins, nuclear or plastid DNA (Bekele, 1983; Brown *et al.*, 1990; Liu and Furnier, 1993). The total genetic variation within each species can be partitioned into individual within and among population, and species levels. The stochastic forces and recombination states affect genetic variations at each level as well as the different evolutionary forces such as directional and non-directional selections (Barrett and Kohn, 1991).

The loss of genetic variation has a net effect on the fitness and viability of the various hierarchical levels. At the level of individual, it increases homogeneity and leads to inbreeding depression, which is reflected by fitness of the individual. At population level, often related to adaptation to local environment conditions, can disrupt the local gene pool and can reduce the chance of adaptation to local environmental changes. At the species level,

loss of diverse populations reduces the potential of the species to respond to environmental changes at regional or global scale (Karron *et al.* 1988; Jain, 1994).

The causes for the reduction of genetic variation include random genetic drift and founder-effect acting upon the truncated size of rare and endangered plant species that can be comparable to a population with a bottleneck or a population size lower than the genetic effective number. In addition to these, high level of inbreeding followed by strong directional natural selection of rare alleles in a limited number of environments leads towards homozygous and lower level of heterogeneity (Barrett and Kohn, 1991). The genetic effective number (effective population number) is much smaller in many rare and endangered plant species and it would be worse in smaller population. Continuous reduction in size, as in case of rare and endangered species, can lead to loss of alleles and results in a significant loss of variations (Maruyama and Fuerst, 1985; Hauser *et al.*, 1994; Frankham, 1995) thus leading to extinction.

2. 4 ISOENZYMES ANALYSIS

One of the most common methods to study genetic variation in plants, based on allele that codes for soluble enzymes, is starch gel electrophoresis of proteins. Isoenzymes can be resolved from most plants regardless of their habitat, age (though in most cases a young plant is needed) and size and virtually any plant tissue can be sampled from leaf, roots, pollen grains and callus. So, the technique is very versatile. It is most suited to analyse the genetic variation at the population, subspecies and species level. The results obtained from isoenzyme analysis are comparable with other techniques used to study genetic diversity in organisms. One advantage over the rest may be, that the isoenzymes loci are co-dominant and

heterozygotes can be scored directly (Crawford, 1983; Ramirez *et al.*, 1987; Brown, 1990; Berg and Hamrick, 1997).

Isoenzymes show Mendelian inheritance, most are co-dominant, they rarely exhibit epistatic interaction and allelic frequencies can be calculated from their band phenotype. Therefore, the levels and distribution of genetic diversity within and among population can be compared directly. The isoenzyme data enables to estimate genetic variability within population or species. The parameters are: percentage of polymorphic loci (P), the mean number of alleles per locus (A), mean number of alleles per polymorphic locus (PA), the effective number of alleles at a locus (A_e) and the excepted proportion of heterozygous of loci per individual (H_e) (Hamrick and Godt, 1989). The fixation index that results from excess or deficiency of heterozygotes is determined using Wright's (1951) fixation index for diploid or Nei's (1973, 1978) can be applied for multiple allele system to compare genetic diversity within population, among population and species. The Wright's (1951) fixation index indicates level of heterozygosity at population level (F_{IS}), among population (F_{IT}) and among species (F_{ST}). In Nei's genetic diversity statistics (Nei, 1973), the total genetic diversity (H_T) is partioned within population (H_S), among population (D_{ST}) and species (G_{ST}) averaged over all polymorphic loci.

The measurement of genetic variation depends on the extraction buffer, homogenisation process, electrolysis buffers, interpretation of the band phenotype and the electrophoresis methods including current, and the buffer conditions (Kephart, 1990). The structure of proteins does affect the appearance of zymogram. The protein, which makes up the enzyme molecules, can have a primary, secondary, tertiary or quaternary structure. The number of protein molecules can be one, two, three, or more, which are encoded by one locus or

multilocus. The bands are referred as monomeric, dimeric, tetrameric or multimeric. Under electric field, the individual polypeptide exhibits different movement depending on its charge and mass. This is exhibited as band phenotype either singly or combined with the rest of the polypeptide molecules that constitute the enzyme. This gives homomeric or heteromeric band features, which may be interpreted as homozygous or heterozygous genotypes. The heterodimers contain two bands for monomeric enzyme, three bands for dimeric, five bands for tetrameric or multiple number of bands for polymeric enzyme (Wendel and Weeden, 1990).

According to Berg and Hamrick (1997) the type of enzymes that are assayed are less than 50. Most of these catalyse metabolic process, either respiration or photosynthesis. The number of individuals taken to identify the number of genetic variability in plant population ranges from 30 to 50 individuals and 10 to 20 allozymes loci.

2. 5 CHLOROPLAST DNA

Chloroplasts are intracellular plastids, which include DNA and ribosomes. The typical terrestrial plants chloroplast DNA (cpDNA) is a circular molecule and the size ranges from 120 kb to 160 kb. It varies little in size, structure and gene content among Angiosperms. The circular DNA is characterised by two large, ca. 25 kb inverted repeats that divide the remainder of the genome into one large and one small single copy region (Sugiura, 1989). According to Palmer and Thompson (1982) and Raubeson and Jansen (1992), all conifers and one group of the legume family have substantially smaller chloroplast genome in which on copy of the inverted repeat is missing and in the parasitic plant *Epifagus* (Orobanchaceae) a massive deletions including a loss of numerous genes has been reported by dePamphilis and Palmer (1990). The changes in gene order and content in the chloroplast genome are rare and

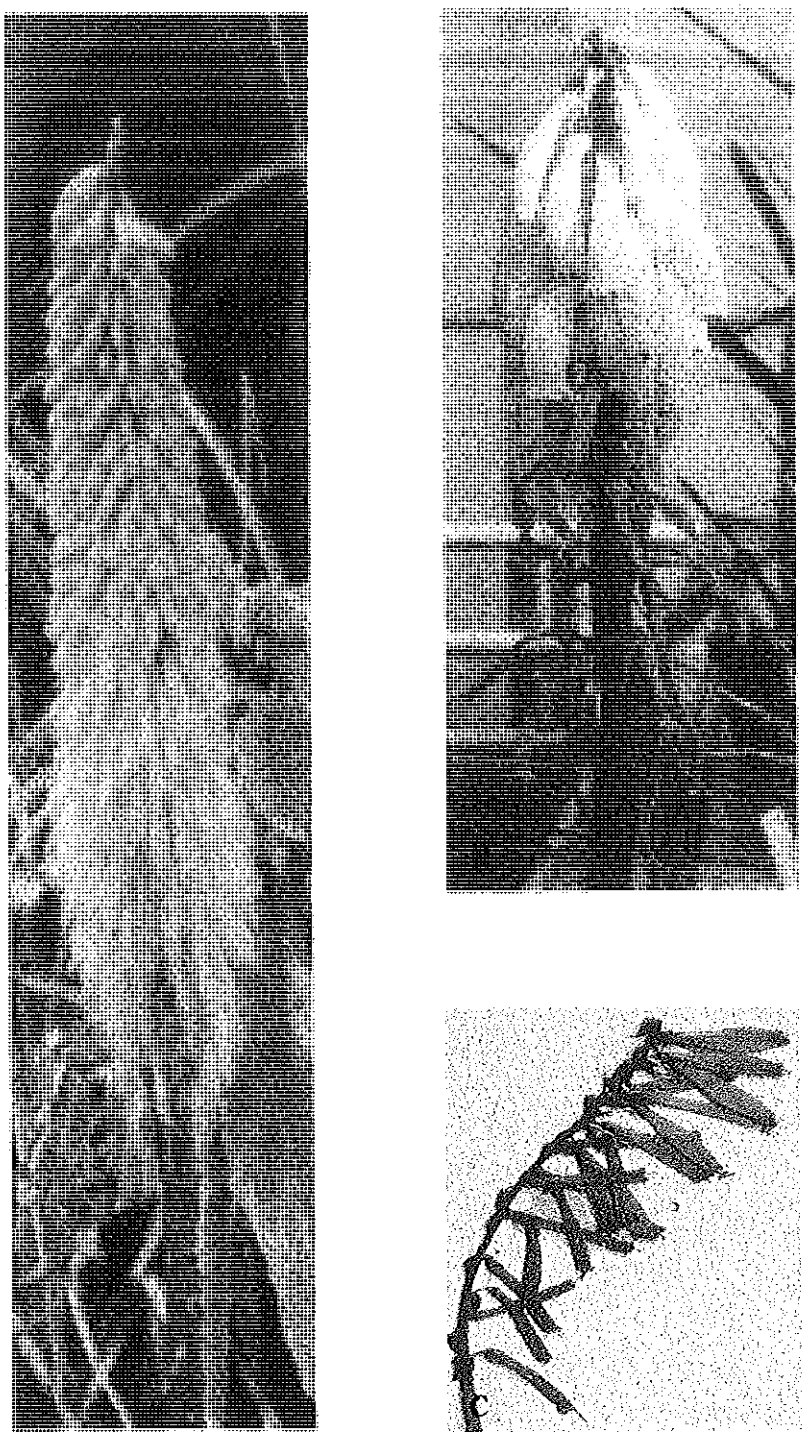

Figure 15. Inflorescences of the endemic *Kniphofia* species: (A) *Kniphofia foliosa*, (B) *Kniphofia insignis* and (C) *Kniphofia hildebrandtii*.