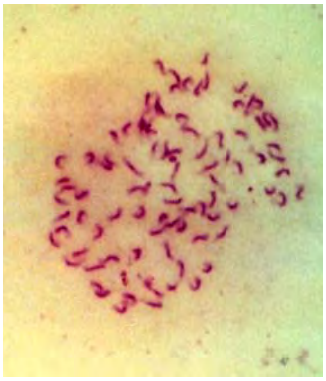
ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF BIOLOGY

Chromosome Study of Local Farmers' Varieties of *Opuntia ficus-indica* (L.) Mill. (Cactaceae) from Tigray, Northern Ethiopia





A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Biology (Applied Genetics)

By

Azimitachew Ayele

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Addis Ababa, Ethiopia

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Table of Contents

Contents Page

List of Figures

Figures **Pages**

List of Tables

Tables **Pages**

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Abstract

Opuntia ficus-indica (L.) Mill. is one of the drought resistant plants that play a significant role in rescuing the lives of people and livestock in arid and semiarid regions of the world. In Ethiopia, particularly in Tigray, it is used as food and forage until cereal crops are harvested as well as during drought seasons. Currently, about thirty-four local farmers' varieties of *O. ficus-indica* are locally identified in the Southern and Eastern Zones of Tigray on the bases of their fruit characteristics and cladode morphology. The present study tried to assess the cytogenetic variability among these local farmers' varieties through standard chromosome count method. Other three sample plants of *opuntia* species from Debre-zeyit, Dire Dawa and Addis Ababa were also included in the study for comparison of their chromosome count with that of the specimens collected from Tigray. The results of the study have shown that all the sample plants chromosomally investigated in the study are polyploids. All the local farmers' varieties of *O. ficus-indica*, for which the somatic chromosome spreads were reliably counted, were found to have an octoploid cytotype, with the same somatic chromosome number of *2n = 8x = 88*. The two specimens of *O. ficus-indica* plant, each from Debire-zeyit and Dire Dawa, have also shown the same ploidy level and somatic chromosome number with that of the local farmers' varieties of *O. ficus-indica* collected from Tigray. On the other hand, the specimen of *O. cylinderica* plant collected from Science Faculty Campus of Addis Ababa University was found to have a different ploidy level with different somatic chromosome number of *2n = 11x = 121*.

The fact that all the local farmers' varieties of *O. ficus-indica* included in the present study belong to the same ploidy level indicates that the variations among those local farmers' varieties in fruit characteristics and cladode morphology could not be attributed to the variation in ploidy level. Further chromosome studies were also recommended to cover wider populations of *O. ficus-indica* from different parts of Ethiopia. It was also recommended to investigate the polyploidy nature, mode of seed formation and genetic variability of *O. ficus-indica* using appropriate molecular techniques such as ISSR and AFLP.

Key words: Chromosomes, Local farmers' varieties, *Opuntia ficus-indica*, Polyploidy, Tigray.

Nowadays, in Ethiopia, particularly in Tigray, different initiatives have been undertaken by individuals as well as governmental and non-governmental organizations in order to introduce modern methods of utilizing *O. ficus-indica* plant as source of food in addition to its fresh fruits.

Opuntia ficus-indica is normally grown under diverse environmental conditions, and farmers locally classify *O. ficus-indica* varieties based on their cladode morphology, fruit characteristics such as shape, texture, size, color and palatability (Tesfay Belay *et al.*, 2009), and their differential response to environmental stresses (Fetien Abay, 2003). Such a traditional classification of *O. ficus-indica* varieties may be an ambiguous task. Thus, determining quantitative and qualitative variability of these varieties in terms of their genetic variability either using cytogenetic studies or molecular markers is an ideal approach for the improvement and conservation of plant genetic resources (Wang *et al.*, 1998). Therefore, this thesis is an attempt to study the variability of local farmers' varieties of *O. ficus-indica* in the Northern Ethiopia, particularly in Eastern and Southern Zones of Tigray, based on cytogenetic information.

Such a cytogenetical study would contribute to the conservation of genetic diversity of *O. ficus-indica* and generate information for wide hybridization as well as for the development of new varieties through the application of modern techniques of plant breeding which would contribute to a sustainable food supply and enriched economy of the country.

1. INTRODUCTION

Opuntia ficus-indica is a drought-resistant plant species, taxonomically placed within the genus *Opuntia*, under the botanical family Cactaceae (Mondragon and Bordelon, 1996). It is grown in a number of countries throughout the world. In Ethiopia, it is grown in different regions of the country, but widely distributed in the Northern Ethiopia, particularly in Tigray. The plant grows in most parts of Tigray and it is used for various purposes, such as food, forage, erosion control and live fences around farmlands and homesteads (Fetien Abay, 2003).

The plant *O. ficus-indica* has different names in different languages all over the world. It is known by the name 'prickly pear' or 'barbary fig' or 'indian fig' or 'opuntia' in English, 'tuna' or 'nopal' in Spanish, 'nochtli' in Nahuatl language of Mexico (Valdez *et al*., 2001), and 'beles' or 'qulqwal bahri' in Tigrigna, 'gura' or 'hadaamii' or 'tini' in Afan Oromo or 'qulqwal' or 'yeberha qulqwal' in Amharic here in Ethiopia (FEE, 2000). The common name, cactus comes from the Greek word "kaktos", meaning a "prickly plant" (Defelice, 2004). Members of the family Cactaceae are an exciting and challenging group of plants because of their diverse morphology, their adaptations to the arid environment, and their versatile reproductive strategies (Rebman and Pinkava, 2001).

Although *O. ficus-indica* has a diverse economic importance in different parts of the world, only little research has so far been done on its genetic resources. To date, in Ethiopia, only a few researches have been done on the economic importance and productivity of *O. ficus-indica* through the data collected from local farmers and few studies done by domestic researchers (Mitku Haile *et al*., 2002). Relatively, more researches have been done on it in Mexico, and most of these studies indicated that *O. ficus-indica* is extensively used as food and medicinal plant by the people of Mexico and Mexican descents, living in the United States of America (Vigueras and Portillo, 2001).

2. LITERATURE REVIEW

2.1. Taxonomic position of *O. ficus-indica*

Opuntia ficus-indica formerly called prickly pear, is a dicotyledonous plant belonging to the genus *Opuntia*, under a monophyletic family Cactaceae (Mondragon and Bordelon, 1996; Mondragon, 2001a; Perez, 2001), which is placed under the botanical division Magnoliophyta, class Magnoliopsida and order Caryophyllales, (Wallace and Gibson, 2002), along with the other betalain-producing angiosperm families, such as Achatocarpaceae, Aizoaceae, Amaranthaceae, Basellaceae, Chenopodiaceae, Didieriaceae, Nyctaginaceae, Phytolaccaceae, and Portulacaceae (Rebman and Pinkava, 2001).

The genus *Opuntia* includes about 200 of the 1,500 species in the family Cactaceae (Hunt, 2000). A significant gap exists in understanding the taxonomy and evolutionary relationship among the members of family Cactaceae due to the relatively little emphasis so far made on systematics of *Opuntia* species (Wallace and Gibson, 2002). Moreover, a number of reasons, such as the existence of hybridization and polyploidy as common features of *O. ficus-indica* plant, and a high degree of phenotypic plasticity of the plant have made it difficult to define the boundaries of species within the genus (Reynolds and Arias, 2001; Wallace and Gibson, 2002; Defelice, 2004).

The difficulties of morphological interpretation have led to publication of a large number of species names in the genus *Opuntia*; many of which are synonyms, or incorrect attributions (Labra *et al.*, 2003). The reasons for such incorrect classification have originated from its large family size, that makes it difficult to identify all members of the family coupled with the above constraints as well as its poor representation in herbaria due to the difficulties in drying and preserving succulent cladodes (Hunt, 2000), and convergent evolution of morphological features in independent taxa (Gibson and Nobel, 1986). These make morphological and genetic information of *Opuntia* to be limited, and the systematics of the genus is unclear (Hardisty and Caire, 1989), and that is why the taxonomy of species belonging to the genus *Opuntia* is still a matter of debate (Labra *et al.*, 2003).

2.2. Botanical description of *O. ficus-indica*

2.2.1. Morphology

Opuntia ficus-indica is a perennial plant characterized by flattened stem segments; each stem is termed as cladode (Wallace and Gibson, 2001). The plant possesses a photosynthetic succulent stem, bearing spines and glochids on modified axillary buds called areoles (Figure 1) (Mondragon and Bordelon, 1996), but lacking broad green leaves (Wallace and Gibson, 2002). In addition, tiny cylindrical or conical succulent and ephemeral leaves are found only on young *O. ficus-indica* stems (Mondragon, 2001a). The ephemeral leaves are replaced by spines and glochids at maturity, and these are considered as modified leaves enabling the plant to withstand drought with a reduced rate of transpiration (Firew Tegegne, 2001).

Figure: 1. Morphological parts of *O. ficus-indica* plant. Cladodes are modified stems, bearing areoles from where spines arise. (Photograph captured from TARI orchard)

Even though anatomical study of matured cladodes, in both wild and cultivated *Opuntia* species, growing in different environments revealed the morphology like that of other seed plants; the shoots consist of internode, node and axillary buds (Salgado and Mauseth, 2002). Plants of *O. ficus-indica* derived from seeds tend to grow in upright slender manner, branching

this species, the external tissue which is the epidermis is composed of compact cells, some of which produce root hairs (Dubrousky and North, 2002).

2.2.2. Physiology

Opuntia ficus-indica is a Crassulacean Acid Metabolism (CAM) plant having high water use efficiency to grow in arid and semi-arid regions (Nobel and Bobich, 2002). The water use efficiency of *O. ficus-indica* is three to five times greater than that of C_3 and C_4 plants (Nobel, 2001). This is because of stomatal opening of CAM plants occur predominantly at night when air and plant temperatures are lower (Nobel, 1995; Nobel and Bobich, 2002). Succulent CAM plants fix CO_2 in the dark and store it as malic acid (Lee, 2010). The stored malic acid is metabolized back to CO_2 during the day time for photosynthesis, although the stomata close during the day to preserve water (Rebman and Pinkava, 2001). In addition, *O. ficus-indica* plant has a thick cuticle that reduces water loss and massive photosynthetic stems act as a water reservoir (Nobel, 2001).

The stem tissues of cacti often contain large quantities of complex polymeric carbohydrate mucilage, which is hydrophilic and affects water relations (Gibson and Nobel, 1986). The mucilage involves in wound healing of the plant. When it is exposed to air, it dries quickly, covering the wound as well as reducing the water loss and the possibility of infection (Mondragon and Bordelon, 1996). An important feature common to most cacti is the relatively high levels of calcium content in the form of calcium oxalate accumulated in the epidermal cells of the plant that results in compact cells of its epidermal tissues (Salgado and Mauseth, 2002).

2.2.3. Reproductive biology

The reproductive versatility is extremely widespread in the members of the genus *Opuntia*, and it can play an important role in the ecological adaptation of the plant to aridity (Pimienta and Castillo, 2002; Reyes *et al.*, 2006). Despite the fact that, the reproductive potential of *Opuntia* species varies with the species and cultivars, both sexual and asexual reproductions are possible in *O. ficus-indica* plants. Commercial orchards use vegetative propagation while the sexual propagation is used only for breeding purposes (Mondragon, 2001a).

2.2.3.1.Asexual propagation

The most common means of vegetative propagation in *Opuntia* species is through the use of cladodes, which bear large number of areoles (Santacruz *et al.,* 1998). Vegetative propagation is possible with pieces of cladodes, whole cladode segment or short branches (Mondragon and Bordelon, 1996). The distinctive vegetative structure of cacti from which spines, glochids, flowers and adventitious roots are developed, is the areole (Pimienta and Castillo, 2002). However, new propagules can be obtained from almost every part of the plant, including flowers and unripened fruits (Mondragon, 2001a).

The planting material for vegetative propagation, most often are cladode cuttings of one year old from healthy and vigorous plants (Mitiku Haile *et al.,* 2002). Cladodes should be cut at their joint to the parent plant and the base of the cuttings is disinfected with Bordeaux mixture (1 kg copper sulfate, 1 kg calcium hydroxide in 100 liters of water) to control bacterial rot infection at the cut surface (Lopez *et al.,* 2001). Cladode cuttings are again allowed to be dehydrated for 4-6 weeks in a dry shaded place before being planted (Inglese *et al.,* 2002) so as to heal the wound around the cut surface (Mondragon *et al.,* 2001; Mitiku Haile *et al.,* 2002). A new plantation of *O. ficus-indica* can start to give yield after one to four years, depending on the environmental condition, cultivar type, and the number of cladode segments used for planting (Mondragon and Perez, 2002).

Several *Opuntia* species, including *O. ficus-indica* have been reported to exhibit apomixis (Stebbins, 1971; Mondragon and Pimienta, 1995), that enables them to generate asexual seedlings from the maternal tissue (Mondragon, 2001b; Mondragon and Perez, 2001). A molecular study conducted by Mondragon (2001b), revealed that the RAPD banding patterns of seedlings emerging late were identical with that of their maternal origin, and such a result is one of the major indicators for the presence of apomixis in *Opuntia* species. Other indicators for apomixis are, unusual high fertility of aneuploids, triploids, wide crosses or other plants expected to be sterile, and multiple seedlings per seed (Singh, 2003b).

2.2.3.2.Sexual propagation

Sexual reproduction promotes active gene exchange and helps to maintain genetic variability (Rebman and Pinkava, 2001). Like other sexually reproducing angiosperms, *O. ficus-indica* plants produce flowers (Mondragon and Perez, 2001). Flowers of *Opuntia* species are hermaphrodite (Reyes *et al.*, 2006) with inferior ovary and flower color that varies from yellow to reddish (Pimienta and Castillo, 2002). Particularly, *O. ficus-indica* has flowers with yellow color, which may fade into pink color after pollination has taken place (Mondragon, 2001a). The flowers open at day time and close in the evening; flowers' opening, typically, lasts 8 to 11 hours (Pimienta and Castillo, 2002). The time between flower bud differentiation and flower opening is relatively short for cacti, and about 40 to 50 days for *Opuntia* species (Pimienta and Castillo, 2002). Flowering season varies according to climatic conditions, cultivar types, and crop management (Mondragon, 2001a). Under normal conditions, *O. ficus-indica* plant flowers from March to April in the Northern hemisphere, and from September to October in the Southern hemisphere (Nerd and Mizrahi, 1997). Self-pollination commonly occurs in *O. ficus-indica* (Mondragon, 2001a) due to the early shedding of the pollen grain before the flower opens (Mondragon and Bordelon, 1996). But it is not the only mode of pollination, as cross pollination also occurs by means of insects, mainly bees (Mondragon, 2001a). The stigma of most cacti flowers shows characteristics suitable for insect landing and the stamens show thigmotrophic sensitivity (Pimienta and Castillo, 2002). Fruits of *O. ficus-indica* develop from both the ovary of the flower and the receptacle that surrounds the ovary (Gibson and Nobel, 1986), and the fruit reaches full maturation between 98 and 112 days after flowering (Duru and Turker, 2005).

Hybridization in *Opuntia* species under natural conditions is very high (Mondragon and Perez, 2001; Reyes *et al*, 2006). This can be confirmed by the report that all Mexican cultivars are products of hybridization of *O. ficus-indica* with different wild *Opuntia* species (Pimienta, 1995). Hybridization of *O. ficus-indica* was first claimed by Luther Burbank at the beginning of the 20^{th} century, which in turn, led to the development of the spineless cactus (Mondragon and Bordelon, 1996). Out-crossing occurs commonly among cacti due to self-incompatibility, dichogamy, herkogamy and unisexuality, (Pimienta and Castillo, 2002).

Sexual propagation of *O. ficus-indica* plants from seeds may take prolonged time due to the hard lignified integuments and funiculus of the seeds that obstructs radicle protrusion during germination (Altare *et al.*, 2006). Seed germination of *Opuntia* species may start after a week or may extend up to two months following sowing depending on the seed conditions, as well as species and cultivar types (Mondragon and Perez, 2001).

2.2.4. Ecology

Opuntia species and cactus plants in general, are widespread and are adapted in many diverse habitats. They occupy areas where there is little competition from other plants, particularly when growing under extreme conditions (Rebman and Pinkava, 2001). The plant *O. ficus-indica* is well adapted to arid and semi-arid regions with annual rainfall of 200 to 250 mm, but its commercial cultivation requires the minimum of about 450 mm annual precipitation (Pimienta, 1995). The plant is sensitive to freezing temperature, but extremely tolerant to high temperature (Nobel, 2001; Nobel and Bobich, 2002). The best temperature for *Opuntia* production ranges between 18 – 29 ^{0}C (Azocar, 2001). It can also grow within 6 ^{0}C - 36 ^{0}C annual minimum and maximum temperature, respectively with 1,800 and 116 mm annual rainfall. In regions with more than 1,800 mm annual rainfall, *O. ficus-indica* plants can be grown but their susceptibility to fungal and bacterial diseases would increase (Gallegos *et al.*, 1995). There are reports that the prickly pear can be cultivated in the range of 800 to 1,800 meters above sea level, and also grows outside of this altitude range (Vazquez and Gallegos, 1995).

Opuntia species grow over a wide range of soil types (Inglese *et al.*, 2002), and they perform well on deep, light textured soils, including coarse sands but not on clay soils (Kock, 2001). They can also grow on very thin and poor soils (Fuentes and Murillo, 1996). Soil with poor drainage, a high water table or superficial impermeable layer should not be used for planting *Opuntia* plants. Like other most cacti, *O. ficus-indica* is sensitive to soil salinity, with 50 to 70 mM sodium chloride being upper threshold for its successful growth (Inglese *et al.*, 2002). The roots are highly affected by salinity than do its shoots (Nobel, 2001). *Opuntia* plant growth is inhibited by shading (Mondragon *et al.*, 2001), water logging and alkali soil (above pH 8.5) (Kock, 2001). Photoperiod length and carbondioxide concentration have been recognized as

important factors that affect the growth of cacti and succulent plants as a whole (Mondragon, 2001a).

Opuntia species show high phenotypic plasticity that react to differences in their environments more quickly and with more drastic growth changes than do other cacti (Mondragon, 2001a). The versatile reproductive systems of *Opuntia* species are often controlled by environmental factors, suggesting that their genetic systems may exhibit phenotypic plasticity (Pimienta and Castillo, 2002).

The ability of *O. ficus-indica* to adapt to different pedoclimatic environments has allowed the plant to be cultivated in many countries such as Europe, America, Asia and Africa (Basile, 2001). This wide diversity of habitat varying from extremely dry deserts to lush tropical rainforests has resulted in various *Opuntia* plant morphology and genetic diversity (Anderson, 2001).

2.3. Origin and geographic distribution of *O. ficus-indica*

Opuntia ficus-indica is a xerophytic plant native to central Mexico and the Caribbean region (Wallace and Gibson, 2002). It is a fruit crop which is important in agricultural economies throughout arid and semi-arid parts of the world (Griffith, 2004). It has been farmed for thousands of years together with those oldest cultivated plants in Mexico such as corn, beans and agave (Mondragon and Bordelon, 1996; Mondragon, 2005). It is proposed that *O. ficus-indica* is a domesticated form of *O. megacantha* (Labra *et al.*, 2003), and the highlands of Mexico are the center of genetic diversity for all *Opuntia* species (Mondragon and Pimienta, 1995; Griffith, 2004).

Opuntia ficus-indica plant was introduced to Europe by the Spanish conquerors in the late 15th and early 16th centuries (Barbera *et al.*, 1992; Defelice, 2004). It spread to most of the Mediterranean countries after the 16th century (Aounallah *et al*, 2005), and these countries are expected to have cultivated varieties all belonging to a single species, which is *O. ficus-indica* (Pimienta, 1995). North Africa and Sicily were the first regions that introduced and farmed prickly pear next to southern Spain due to their geographical contiguity or social and political relationships with the Spanish Peninsula (Pimienta, 1995; Mondragon and Bordelon, 1996). It

probably reached the rest parts of Africa, Asia and Australia through birds that fed on its fruits, and its shipment as anti-scurvy food during European colonization (Casas and Barbera, 2002; Mondragon, 2005).

Opuntia ficus-indica is currently cultivated in more than twenty countries of the world for fruit production in both hemispheres and all continents, except Antarctica (Nobel, 1995; Inglese *et al.*, 2002). Even though the largest number of germplasm collection and entries are located in Mexico, where the greatest diversity occurs for indigenous *O. ficus-indica* (Chapman *et al.*, 2002), the domesticated *O. ficus-indica* nowadays, exists in Brazil, Colombia, Peru, Bolivia, Argentina, Chile, Greece, Italy, Turkey, Yemen, Syria, Lebanon, Israel, Egypt, Tunisia, Algeria, Morocco, Ethiopia, Eritrea, South Africa and even as far as inland China (Inglese *et al.*, 2002). Madagascar and Australia also have large areas of wild *O. ficus-indica* plantation (Mondragon, 2005).

According to recent field survey conducted on the variability of *O. ficus-indica* in these main producing countries, the arid lands of Mexico host the largest phenotype variability of the specie in the world (Pimienta, 1995). Mexico and Italy are the main producing countries of *O. ficus-indica* (Mondragon, 2001a). The most important edible *Opuntia* species in wild and cultivated populations of Mexico are *O. amyclaea* Tenore, *O. ficus-indica* Mill., *O. hyptiacantha* Weber, *O. leucotricha* De Candolle, *O. lindheimeri* Engelm, *O. megacantha* Salm-Dyck, *O. robusta* Wendland, *O. streptocantha* Lemaire and *O. tapona* Engelmann (Soberon *et al.*, 2001; Vigueras and Portillo, 2001). Of these, *O. ficus-indica* is the most abundant and representative species in the natural population (Pimienta, 1995).

2.3.1. Geographic distribution of *O. ficus-indica* in Ethiopia

Opuntia ficus-indica plant or ‘beles’ was introduced to the Horn of Africa around 1846 and 1887 by the Italian and German Missionaries (Vigueras and Mondragon, 2005). According to Fesseha Yaye (2010), it was assumed as the catholic missionaries brought it to Ethiopia 160 years ago through the northern part of the country, particularly through ‘Alitena’ in Woreda ‘Erob’, and widely distributed in the arid and semi-arid regions of Tigray, in Northern Ethiopia. It was also supposed as the plant was also introduced to Southern Zone of Tigray in

only in the upper cladodes, whereas plants grown from cuttings tend to have thicker and wider cladodes as well as more pads on the lower parts of the plant (Chapman *et al.*, 2002).

The basic meristematic units in *Opuntia* species are the areoles (Figure 1). They are arranged in clusters and positioned in a helicoidal fashion on the cladodes and develop either into branches or flowers (Mondragon and Bordelon, 1996; Mondragon *et al.*, 2001). *Opuntia ficus-indica* plants produce fruits that have thick rinds containing areoles and comparatively large seeds covered by hard, bony, light colored arils (Hunt, 2000). The fruit is an oval, elongated berry, with a thick pericarp and a juicy pulp (Figure 2) (Piga, 2004). The peel is dark green until 50 days after anthesis; between 60 to 70 days, the peel starts to become colored and the pulp starts to acquire the characteristic color of the cultivar (Reyes *et al.*, 2006).

Figure: 2. Fruit colors of *O. ficus-indica* plant. A) Red-purple, B) Yellow-orange, and C) White-cream or greenish (Source: Inglese and Nefzaoui, 2003).

Opuntia ficus-indica plants contain different pigments that are responsible for different fruit colors of different cultivars (Saenz and Sepulveda, 2001). The pigment betalain occurs in purple fruits, betacyanin in red fruits, betaxanthin and chlorophyll respectively present in orange and green fruits (Piga, 2004). The presence of pectin substances in *O. ficus-indica* plant is partially responsible for its fruit pulp viscosity (Saenz and Sepulveda, 2001).

Opuntia ficus-indica plants also have shallow roots; usually grow no deeper than 15 to 30 centimeters below the soil surface. The radial pattern of the primary root structure in *O. ficus-indica* plant does not significantly differ from that of most other dicotyledonous species. For

Plantation of the *O. ficus-indica* in Tigray is also broadly grown in the Southern and Eastern Zones of the Region. Its invasive effect has been seen in some areas of Southern Zone of Tigray to the extent that it has been considered as a curse for farming (Mitku Haile *et al*., 2002). *Opuntia ficus-indica* coverage of Tigray reaches about 30,520 ha, and it accounts for 1.8 percent of the total area of the Region (Firew Tegegne, 2001). In most parts of the Region, it is growing wild, but some farmers in the Eastern Zone of Tigray have maintained *O. ficus-indica* orchards in their backyards (Tesfay Belay *et al*, 2009).

Despite the fact that, *O. ficus-indica* is widely distributed and extensively used in Tigray for food, forage, erosion control, and live fences (Fetien Abay, 2003), no special attention has been given to its cultivation by the farming community, and cultivation of the plant is taken as extra time activity, usually done after the completion of harvesting cereal crops. The scientific communities and researchers have also done little on its systematic study to identify and characterize the existing varieties of the *O. ficus-indica* in the Region (Mitku Haile *et al*., 2002).

Cultivars often contain unique assemblages of genes that may be useful for future breeding efforts (Inglese *et al*., 2002). *Opuntia ficus-indica* cultivars for fruit production can be distinguished by the color of the fruit peel and the ripe flesh, which are red-purple, yellow-orange, white-cream or greenish (Figure 2) (Mondragon and Pimienta, 1995). Cultivars also differ in plant shape, cladode and fruit morphology, vigor, fertility, fruit seed content and ripening time (Pimienta, 1995). In Tigray, the local varieties of *O. ficus-indica* are identified by the local farmers, based on taste, size, shape, color, seed count, and peel thickness of the fruits as well as their cladode morphology (Fetien Abay, 2003; Tesfay Belay *et al.*, 2009).

On the basis of the presence or absence of spines on the cladodes, *O. ficus-indica* plants can be classified into two major groups, spiny and spineless varieties (Figure 4). In Tigray, the spineless variety was reported to have better advancement over the spiny one in respect to its pad size, number of branches per plant, and fruit productivity per pad, but it yields fruit only once in a year. The area coverage of the spineless *O. ficus-indica* is less than that of the spiny one because of its vulnerability to be grazed easily by animals. The spiny variety has narrow pads with strong spines, but it possesses good traits such as fruit production twice in a year,

Northern Ethiopia from the Middle East by Muslim pilgrimage around 1920 (Habtu, 2005; cited in Tesfay Belay, 2010). Currently, the plant is found in different parts of Ethiopia lie between 1400 – 2600 meters altitude, in exposed fields and near cemetery around Northern Wello in the Amhara Region (De Bac, 2010), and in some other Regions of the country around Debre-zeyit, Dire Dawa, Hararge, Bale, Arsi and Awassa due to various agents that are hypothesized for its multi-directional spreads (Hunt, 2000).

2.3.1.1. Local farmers' varieties of *O. ficus-indica* in Tigray

Opuntia ficus-indica plantation in Tigray is expanding to areas that are characterized by drought conditions, erratic rainfall, and poor soils subjected to erosion (Mitku Haile *et al*., 2002; Vigueras and Mondragon, 2005). *Opuntia ficus-indica* is predominantly distributed in Tigray more than other parts of the country due to the rugged, stony and sloppy topography of the region that enforce local farmers to introduce the plant around their marginal fields and homesteads for multi-purposes (Figure 3).

Figure: 3. *Opuntia ficus-indica* plant grown around homestead in Kihen Village, about 25 km north of Mekelle. (Photograph captured during field visit)

and better drought tolerance that enables it to give good yield with less rainfall (SAERT, 1994; cited in Mitku Haile *et al*., 2002).

Figure: 4. The two major varieties of *O. ficus-indica* plant. A) Spiny, and B) Spineless varieties. (Photographs captured from TARI orchard during sample collection)

A study conducted by Tigray Agricultural Research Institute (TARI) (2009), has reported about thirty-four local farmers' varieties of *O. ficus-indica* that have been identified in selected sites, found in Southern and Eastern Zones of Tigray. Most of these local varieties showed a minimum variation in their cladode morphology, but they showed significant variability in fruit characteristics such as fruit shape, size, taste, pulp color, seediness and peel thickness (Tesfay Belay *et al.*, 2009). These locally identified farmers' varieties were also kept in a germplasm nursery established by TARI at Mekelle. Those local varieties of *O. ficus-indica* collected from three different Woredas of Tigray are presented below in Tables 1, 2 and 3.

2.3.1.1.1. Farmers' varieties of *O. ficus-indica* from Woreda "Mekhoni"

Based on the data collected from local farmers in the Region, twelve local farmers' varieties of *O. ficus-indica* have been identified from Woreda "Mekhoni" (Table 1), in Southern Zone of Tigray (Tesfay Belay *et al.*, 2009), located about 60 km south of Mekelle and 20 km east of

Maichew. Woreda "Mekhoni" lies between 1500 to 1800 meters altitude with an average annual rainfall of 488 mm (Habtu Lemma *et al.*, 2010), and it is representative of tropical highlands of Southern Tigray in which *O. ficus-indica* has become invasive (Brutsch, 1997).

Table: 1. Local farmers' varieties of *O. ficus-indica* identified from Woreda "Mekhoni"

No.	Local farmers' varieties name	Description	Cladode size		Fruit Wt. (gm)	Fruit color
			L	W		
1	Megal Hailu	Refers to large fruit size	31.5	16.5	92.7	Lemon yellow
2	Tsaeda Aona	Refers to white fruit color and location	33.8	17.1	58.0	Light green
3	Shenkor	Refers to sweet fruit taste	26.8	16.9	66.9	Light green
4	Berbere Hailesilassie	Refers to name of a person	28.1	17.3	54.1	Light green
5	Limo	Refers to spineless cultivar	27.9	14.4	74.6	Pink
6	Meskal Gadaho	Refers to a location where it grows	31.7	17.1	63.3	Pastel green
7	Chewchawe	Refers to salty fruit taste	28.3	16.1	66.5	Pale yellow
8	Tesemsema	Refers to a lovely fruit taste	32.6	16.6	72.9	Pale yellow
9	Mot Kolea	Refers to a location where it grows	39.2	20.3	63.8	Cream
10	AwKulukual Bahri	Unknown	32.3	17.3	52.3	Pale yellow
11	Cheguar	Refers to high glochid abundance	-	-	90.6	Orange
12	Fukur Rosso-Adem	Refers to name of a person	-	-	-	-

Source: Tesfay Belay *et al.*, 2009; Wt = weight, L = length, and W = width.

All the local farmers' varieties identified in Woreda "Mekhoni" are spiny, except the local farmers' variety "Limo", and their cladodes vary from ovate to elliptical shape (Figure 1 and 4). Most of these local farmers' varieties of *O. ficus-indica* also produce fruits with an ovoid shape (Figure 2) (Tesfay Belay *et al*., 2009).

2.3.1.1.2. Farmers' varieties of *O. ficus-indica* from Woreda "Ganta-afeshum"

Nine local farmers' varieties of *O. ficus-indica* have also been identified from Woreda "Ganta-afeshum" (Table 2), in Eastern Zone of Tigray, which is located about 115 km north of Mekelle. Woreda "Ganta-afeshum" is found at an elevation of 2457 meters above sea level with an average annual rainfall varies between 300 and 400 mm (Tigray Livelihood Zone Report, 2007). Most of the local varieties of Woreda "Ganta-afeshum" have ovate cladode shape, except some varieties that have elliptic or rounded cladodes (Tesfay Belay *et al.*, 2009).

Table: 2. Local farmers' varieties of *O. ficus-indica* identified from Woreda "Ganta-afeshum"

No.	Local farmers' varieties name	Description	Cladode size		Fruit Wt. (gm)	Fruit color
			L(cm)	W (cm)		
1	Finshir	unknown	35.5	32.7	106.2	Not recorded
2	Gorzeme	unknown	35.7	21.0	119.7	
3	Keyih Beles	Refers to red fruit color	38.8	21.6	142.2	
4	Koremele	Refers to a candy- like fruit taste	39.7	21.4	67.4	
5	Lemats Beles	Refers to spineless variety	34.1	19.2	140.2	
6	Machuch	Refers to sour fruit taste	29.3	20.2	73.5	
7	Sanguni	unknown	36.4	21.2	133.8	
8	Tsaeda Beles	Refers to white fruit color	31.1	20.5	145.6	
9	Beles Aboy-Halibo	Refers to name of a person	34.5	19.8	121.0	

Source: Tesfay Belay *et al.*, 2009; Wt = weight, L = length, and W = width.

2.3.1.1.3. Farmers' varieties of *O. ficus-indica* from Woreda "Erob"

Eastern zone of Tigray again hosts other thirteen local farmers' varieties of *O. ficus-indica* identified from Woreda "Erob" (Table 3) (Tesfay Belay *et al.*, 2009), which is about 175 km north of Mekelle and closer to the Eritrean border in the northeastern Tigray. Woreda "Erob" lies in an altitude ranging between 1200 and 3000 meters with annual rainfall that varies between 140 and 400 mm (Mulugeta Gebresilassie *et al.*, 2010).

Table: 3. Local farmers' varieties of *O. ficus-indica* identified from Woreda "Erob"

No.	Local farmers' varieties name	Description	Cladode size		Fruit Wt. (gm)	Fruit color
			L(cm)	W (cm)		
1	Gerao	Refers to sweet fruit taste	35.0	19.6	144.4	Yellow orange
2	Nahrisa	Refers to unpleasant/ nausea fruit taste	48.0	23.0	146.5	Pale yellow
3	Kalamile	Refers to colorfulness of the fruit	31.0	21.3	84.5	Pink
4	Adomulhata	Refers to white fruit color with salty taste	33.3	16.0	90.3	White
5	Geleweiti	Refers to a fruit with mixed peel color	33.0	19.3	60.0	White green
6	Gerwanlyele	Refers to watery fruit taste and location	36.6	21.6	109.3	Yellow
7	Suluhna	Refers to spineless cultivar	39.3	17.0	142.3	Yellow orange
8	Hiraydaglayele	Refers to watery taste and location	33.6	21.6	77.4	Yellow
9	Neitsi	Refers to white fruit color	40.3	23.0	114.6	White
10	Orgufa	Refers to early fruit shedding	32.3	15.6	110.9	Yellow red
11	Ameudega Adobelesa	Refers to white fruit color and location	30.0	20.0	97.1	White
12	Hawawisa	Refers to ripen fruit appearance before ripening	34.6	17.3	119.9	Yellow red
13	Asa Kurkura	Refers to red fruit color with round shape	32.6	16.0	54.0	Red

Source: Mulugeta Gebresilassie (2007), M.Sc. thesis; Tesfay Belay *et al.* (2009).
Wt = weight, L = length, and W = width

All the local farmers' varieties of *O. ficus-indica* that were identified in Woreda "Erob" possess cladodes with ovate shape, except the local farmers' varieties "Netsi", "Sulhuna" and "Hawawisa" which have elliptical shaped cladodes. These local varieties produce fruits with ovoid shape, except some varieties like local farmers' variety "Nahrisa" with oblong fruit shape (Tesfay Belay *et al.*, 2009).

2.4. Uses of O. ficus-indica

Opuntia ficus-indica is an emergent crop in regions where recurrent droughts have occurred. Its commercial use has evolved since the second half of the 20th century, and the plant was probably originated in central Mexico, the region with the widest *Opuntia* species germplasm variability as well as the large number of uses of *Opuntia* plants (Mondragon, 2005).

One of the main reasons for the current increased global interest towards the *O. ficus-indica* cultivation is due to its diverse economical importance (Fuentes and Murillo, 1996; Mondragon, 2005). It is commonly used as a source of food, forage, fuel wood, cash income, raw material for various industrial products, as live fences and soil conservation purposes (Brutsch, 1997; Mitiku Haile *et al*., 2002).

2.4.1. As food source

Among the 1,500 species of the family Cactaceae (Hunt, 2000), many species produce edible fruits (Inglese *et al.*, 2002). Many species in the genus *Opuntia* have the most relevant role in agriculture as a fruit crop for arid and semi-arid regions of the world (Mondragon and Perez, 2002). *Opuntia ficus-indica*, *O. robusta, O. hyptiacantha* and *O. megacantha* are currently cultivated in different countries as commercial species of *Opuntia* (Felker and Inglese, 2003). The production of fruits for human consumption from *O. ficus-indica,* either from spineless *ficus-inermis* or the spiny *ficus-amyclaea,* is the most classical and widespread practice in the Mediterranean basin (Le Houerou, 2002). *Opuntia ficus-indica* is the only species cultivated for fruit production in South America, United States of America, Mediterranean basin and Africa (Pimienta, 1995).

Nowadays, there are several countries that are growing *O. ficus-indica* plants; of which Mexico and Italy are the main producers as well as consumers of its fresh fruits (Flores, 1995; Mondragon, 2001a). Fruits of *O. ficus-indica* are also sold as a desert fruit in markets of the United States of America, Chile, Brazil, North Africa, Spain and Greece (Singh, 2003a). It has also become the major income and food source for about four months (from May to August) in Tigray, Northern Ethiopia (Fetien Abay, 2003; Vigueras and Mondragon, 2005).

Fruits of *O. ficus-indica* are valuable food sources (Degano *et al*., 1997) that are rich in calcium, potassium, magnesium phosphorous and vitamins (Salim *et al.*, 2009). Moreover, the fruits contain moderately high sugar content. The majority of the sugars are the reducing type, glucose and fructose, contributing to a very sweet taste of the fruit (Saenz, 1996). However, the presence of glochids on the peel and the hard thick seeds in the flesh are the major constraints of enhancement in consumption (Inglese *et al*., 2002). But still, it has played a significant role during drought condition in saving human and livestock life even in those areas where it is considered as curse (Brutsch, 1997; Tesfay Belay *et al*, 2009).

Opuntia ficus-indica stems are used as a source of food in the form of 'nopalito' (Saenz, 2000), which is 20 up to 30 days old tender young cladode (Vigueras and Portillo, 2001) eaten fresh or cooked in various dishes as a green vegetable (Singh, 2003a). This traditional dish is widely used in Mexican cooking, and its use is somewhat restricted to Mexican and groups of Mexican origin residing abroad (Flores and Osorio, 1997). 'Nopalito' is the fifth most important vegetable in volume of production in Mexico next to tomatoes, potatoes, chillis and onions (Flores, 1995). The presence of spines on the cladodes of *O. ficus-indica* is a serious impediment to use the plant extensively as food source (Mondragon and Perez, 2001). In addition to the more obvious use of cactus for food in the form of fresh fruit and 'nopalito', its mucilage is also used in food products to create good mouth taste and to increase the viscosity of the food (McCarthy, 1996).

2.4.2. As forage and fodder

Opuntia ficus-indica plants are again used as source of forage and fodder in arid and semi-arid areas (Le Houerou, 2002). Therefore, they are planted around homesteads and in the

rangelands as fodder bank for drought seasons (Brutsch, 1997). Cactus, specifically *Opuntia* species have broadly been exploited as useful livestock forage during drought seasons, primarily, by providing digestible energy, water and vitamins (Reynolds and Arias, 2001). The use of cladodes as well as fruit peels of *O. ficus-indica* for the purpose of forage began with the colonization of North Mexico by the Spaniards in the 16th century (Flores and Osorio, 1997), and currently, it is prevalent in Brazil, Mexico, Tunisia and various other countries such as United States of America (Nobel, 1995) and Ethiopia (Firew Tegegne, 2001).

2.4.3. As raw materials for industrial products

The use of processed food products of *O. ficus-indica* is very low in the majority of this plant producing countries, except Mexico (Saenz, 1996). The most common product made from cladode is marmalade (Hernandez *et al*., 2002). It can also be transformed into flour to replace wheat flour in cookies (Barbera *et al.*, 1992; Saenz, 1996). *Opuntia ficus-indica* is used in improving the quality of food due to its high content of fibers and pectin components of diet (Fuentes and Murillo, 1996). One by-product that can be obtained from *O. ficus-indica* plant is the mucilage. The mucilage obtained from fruit pulp and cladode is commonly described as water soluble pectin like polysaccharide (Cardenas *et al*., 1997), containing rhamnose, arabinose, galactose and galacturonic acid (Saenz, 1996). Pectin is widely used as texturizer and stabilizer in a variety of foods and other industrial products. It occurs in large number of plant species but its commercial source is very limited. Therefore, *O. ficus-indica* can be considered as very promising new source of these biopolymers (Goycoolea and Cardenas, 2003). *Opuntia ficus-indica* can also be exploited as good source of natural additives or substituted materials for production of many foods, like ice-cream and candy (McCarthy, 1996, Saenz, 2000) due to its low acidity, high sweetness, good nutritive value and attractive stable color (Samahy *et al*., 2009). Fruits are also used in the production of alcohol (Barbera *et al.*, 1992), and seeds are used to produce edible oil that has a high degree of unstauration with high linoleic acid content (Saenz, 1996).

Along with its uses as raw material for processed food products, *O. ficus-indica* has been used as potential source of an industrial hydrocolloid gum (Cardenas *et al*., 1997). It has also been

utilized as a raw material in the production of soap, dyes and cosmetics, like shampoo, cream and body lotions (Flores and Osorio, 1997).

2.4.4. For cochineal production

Opuntia ficus-indica stem is also used in the production of carminic acid, which is a natural colorant developed by pre-colonial indigenous people of Mexico (Barbera *et al.*, 1992). Carminic acid is produced by an insect, belonging to the genus *Dactylopius*, which is known as cochineal (*Dactylopius coccus*, Costa) (Vigueras and Portillo, 2001). This insect is a parasite that infects several cacti species of two closely related genera, *Opuntia* and *Nopalea* (Mondragon, 2001a). Therefore, *O. ficus-indica* is advantageously used as a host for cochineal insect that yields the natural red dye (Brutsch and Zimmermann, 1993; Mondragon, 2005).

2.4.5. For medicinal uses

Several studies revealed that *O. ficus-indica* is used as a traditional medicine predominantly in Mexican-American population (Perez, 2001). The cladodes are used as a local anti-inflammatory remedy for edemata and arthrosis, as regulators of smooth musculature in the treatment of whooping cough and as anti-infective agents (Barbera *et al.*, 1992). Its effect in reducing serum sugar level has been reported in a few published studies (Aguilar and Ramirez, 1996). The soluble carbohydrate in cladodes has been examined for blood glucose and insulin lowering properties in type-II diabetic patients (Felker and Inglese, 2003). Consumers have also found out that ‘nopalitos’ are beneficial for weight reduction and reducing sugar and cholesterol level in the blood (Flores, 1995). It is also experimentally proved that the traditional use of cladodes for wound healing is effective (Maria *et al.*, 2003).

2.4.6. For soil conservation and other purposes

Opuntia species are being utilized to prevent soil erosion and to combat desertification (Fuetes and Murillo, 1996). This is because of their great potential of adaptations to grow in extremely degraded soils inadequate for other crops and ideal for responding to global environmental changes (Reynold and Arias, 2001). *Opuntia* species are also important as vegetation cover in arid and semi-arid areas due to their suitability for the recovery of degraded lands

(Mondragon, 2005). They are also used as live fences and windbreaks around farmlands and homesteads (Barbera *et al.*, 1992; Fetien Abay, 2003). Some species of *Opuntia* are also used for decorating farmlands and home gardens (Singh, 2003a). By-products of the *O. ficus-indica* plant are used as organic fertilizers, insecticides and other biotechnological purposes (Fuetes and Murillo, 1996).

2.5. Essential agronomical traits in breeding of *O. ficus-indica*

Understanding of the phenotypic and genotypic variations in *O. ficus-indica* is critical in the development of cultivars either through the conventional breeding or biotechnological approaches, and for future germplasm collection (Arnholdt *et al.*, 2001). In regard to breeding, the need for studies on mode of inheritance of basic traits, such as fruit color, size, shape and seediness along with cladode morphologies are imperative (Mondragon and Bordelon, 1996).

Modern cultivars of *O. ficus-indica* are the product of selections made by the growers (Mondragon, 2001a), and the names of the cultivars reflect fruit or cladode phenotypic characteristics, as well as the plant response to environmental stresses and its resistance to handling (Mondragon and Bordelon, 1996). For instance, *O. ficus-indica* cultivars available in the Sicily area of Italy are "*Gialla*", "*Bianca*" and "*Rossa*" with a fruit pulp color of yellow, white and red, respectively (Barbera *et al*., 1992). In Chile, the cultivar type "*Verd*" with green fruit pulp color is the most commonly cultivated variety of *O. ficus-indica*. In Israel, the cultivar "*Ofer*" with yellow fruit pulp color and in South Africa, the cultivar with light green fruit pulp color are cultivated commonly (Mondragon, 2001a). But still, the largest genetic diversity of *O. ficus-indica* occurs in Mexico, whereas in other countries only a few cultivars have been described and are commercially cultivated (Inglese *et al.*, 2002).

Current interest for the improvement of *O. ficus-indica* cultivars is towards the development of spineless varieties, and varieties with large fruit size, better palatability, less seediness as well as less glochids on the fruit peels (Chapman *et al.*, 2002). Genes for low number of areoles and short glochids are present in *O. robusta* (Mondragon, 2001a), although the fruit of this species is not well accepted due to its low sugar content, bland flavor and short shelf life (Mondragon and Bordelon, 1996). The genetic makeup, long lived perennial habit, and large size of the *O.*

ficus-indica plant make the maintenance of its germplasm bank difficult and costly (Mondragon, 2001a; Chapman *et al.*, 2002).

2.6. Biochemical and molecular studies on *O. ficus-indica*

Most plant molecular geneticists have focused on economically important crop species. However, few researchers like Chessa *et al.* (1997) and Wang *et al.* (1998) had conducted isozyme and RAPD analysis on cultivars of *O. ficus-indica,* respectively. The isozyme analysis by Chessa *et al.* (1997), reported that all the cultivars of *O. ficus-indica* included under the study from the Sicilian germplasm displayed the same banding patterns, revealing lower genetic diversity among *O. ficus-indica* ecotypes and cultivars studied.

Even if DNA isolation from fresh *O. ficus-indica* material is difficult due to the presence of high mucilage content that interferes with the DNA extract (Arnholdt *et al.*, 2001), recent DNA molecular marker data has raised significant questions on the validity of the traditional taxonomic approaches to the classification of the commercial *O. ficus-indica* fruit types (Felker and Inglese, 2003). Despite the fact that the perennial species with their large size, low growth rate and huge genomes have been poor prospects for gene transfer methods (Mondragon and Bordelon, 1996), successful transformation of foreign DNA was demonstrated by means of particle bombardment (Mondragon, 2001a). Moreover, an ongoing research has revealed that the prickly pear may be amenable to gene transformation using *Agrobacterium rhizogenese* (Chapman *et al*., 2002).

2.7. The cytogenetics of *Opuntia* species

Plants of the family Cactaceae have relatively smaller chromosome size with an average length of 2.3 µm (Las-Penas, 2009). Cactus chromosomes have few distinctive morphological characters, and their mitotic chromosomes appear similar (Ross, 1981). The majority of cactus species are diploid with the basic chromosome number of $x = 11$ (Spencer, 1955; Ross, 1981), but fewer cacti species particularly in the sub-family *Opuntioideae* show polyploidy (Rebman and Pinkava, 2001).

Most of the species of sub-family *Opuntioideae* are reported to have polyploid chromosome numbers (Sosa, 1964; Yuasa, 1973; Pinkava *et al.*, 1992; cited in Mondragon, 2001a). Several cytogenetic studies have revealed the existence of a wide range of ploidy levels for *Opuntia* species, 2x, 3x, 4x, 5x, 6x, 8x, 10x, 11x, 12x, 13x, 19x and 20x (Mondragon and Bordelon, 1996). Cultivated varieties of Mexican *O. ficus-indica* were found commonly to have higher chromosome numbers of hexaploid (*2n = 6x = 66*) and octoploid (*2n = 8x = 88*), whereas the wild varieties have diploid (*2n = 2x = 22*) and tetraploid (*2n = 4x = 44*) chromosome numbers (Pimienta, 1995).

Perhaps polyploidy has played a vital role in speciation of the *Opuntia* species (Pimienta, 1995). The great genetic variability of *Opuntia* species might have been arisen via natural hybridization associated with polyploidy and reproductive isolation (Gibson and Nobel, 1986; Wallace and Gibson, 2002). A comparison of the ploidy level with the mode of reproduction in the Cactaceae revealed that polyploidy is more likely to become established in self-fertile or apomictic taxa (Ross, 1981). Several reports have suggested that the differences in fruit and cladode size found in wild and cultivated populations of *Opuntia* species are likely linked to differences in ploidy levels (Mondragon, 2001a).

2.8. Cytogenetics and its significance in plant systematics

Cytogenetic study includes determination of number and morphological characterization of chromosomal complements that are arrested at mitotic metaphase stage through series of treatments. Such studies have revealed that chromosomes of related species may vary in several ways, which are collectively summed as karyotype. These cytogenetic details have been found extremely useful at all levels of the taxonomic hierarchy as well as resolving controversies on biogeography, taxonomy and evolution of different taxa (Stace, 1989).

2.8.1. Karyotype

The term karyotype has been given to the group of characteristics that identifies a particular set of chromosomes (Singh, 2004). These characteristics include chromosome numbers, their relative size, centromeric position, arm length, secondary constrictions and satellites (Verma

and Agarwal, 1974). Karyotypic variation is indispensable as genetic variability, as well as species differentiation (Urdampilleta *et al.*, 2007).

2.8.1.1. Chromosome morphology

The chromosome morphology includes the centromere, secondary constriction, satellite and telomeres of a chromosome (Stace, 1989). The different aspects of chromosome morphology are usually studied at the metaphase stage of mitosis at which chromosomes have become thickened and shortened to the maximum extent, and when they are most easily stained in which the principal landmarks, may be seen (Stebbins, 1971).

2.8.1.1.1. Centromere

Centromere is a constricted regions of chromosomes to which the spindle fibres are attached and involved in chromosome segregation during cell division (Kidwell, 2005). Chromosomes of most organisms contain only one centromere and they are said to be monocentric chromosomes (Singh, 2003b). Unless it is located at chromosome end, the centromere divides a chromosome into two distinct regions, referred to as chromosome arms (Verma and Agarwal, 1974). In a given chromosome, the centromere is localized in one particular region, whereas its relative position varies from chromosome to chromosome (Singh, 2004), and thus the difference in centromeric position among chromosomes is used to classify chromosomes into several categories, such as those indicated below.

Telocentric chromosomes are those which have the centromere at their end and such chromosomes have only a single arm (Stebbins, 1971).

Acrocentric chromosomes are those having the centromere nearer to one end and thus possess one very short arm and another one relatively long arm (Singh, 2003b).

Sub-metacentric chromosomes have a centromere located somewhat away from middle portion of the chromosome and thus forming two unequal arms, but not as extreme as in the case of acrocentric chromosomes (Verma and Agarwal, 1974).

Metacentric chromosomes contain a centromere at or near the middle region and thus form two equal or nearly equal arms (Singh, 2003b).

Alteration in the position of the centromere which converts metacentric to sub-metacentric or acrocentric chromosomes or vice versa occurs through inversion of chromosomal segments containing the centromere or through unequal translocation between non-homologous chromosomes (Thompson, 2005). Metacentric chromosomes can also arise by the Robertsonian fusion of two telocentrics (Sharma and Sharma, 1999). Conversely, horizontal breakage through the centromere of a biarmed chromosome can give rise to two telocentric chromosomes. The latter two types of chromosomal rearrangements change centromeric position, but respectively decrease or increase chromosome number without affecting the total number of arms and total amount of the genetic material.

2.8.1.1.2. Secondary constriction and satellite

Another useful aspect of chromosomal gross morphology is the position of a secondary constriction. Secondary constriction is a thin undercondensed region also known as nucleolus organizer region (NOR) (Singh, 2004). The secondary constriction also delimits distally a condensed chromosome segment that referred to as a satellite. The satellite is connected to the rest part of the chromosome via the secondary constriction (Stace, 1989; Sumner, 2003). Chromosomes possessing the satellite are also designated as sat chromosomes and only one pair or a few pairs of satellited chromosomes are present in diploid chromosomes (Verma and Agarwal, 1974).

2.8.1.2. Genome Size

Genome size refers to the total DNA content of the nucleus of a particular cell of an individual or a species (Stebbins, 1971). The DNA amount in the unreplicated haploid nuclear chromosome complement is known as C-value. Since species vary in their C-values, it can serve as a significant biodiversity feature essential in species characterization (Bennett and Leitch, 2005a).

2.8.1.3. Chromosome size

Chromosome size refers to the entire length of individual chromosomal complements, also determined as relative chromosome size (Stebbins, 1971). Monocots generally possess large-sized chromosomes than dicots. Plants in general have large-sized chromosomes in comparison to animals (Verma and Agarwal, 1974). Chromosomes of woody angiosperms are usually small with little size differences between related species and genera, whereas chromosomes of herbaceous angiosperms show great size difference between different genera of the same family, sometimes even between different species of the same genus (Sharma and Sen, 2002). The chromosomal complements or karyotypes of most plant species consist of chromosomes which are comparable to each other in size. Difference in relative chromosome size can be brought about by unequal translocation, deletion or duplication of chromosomal segment (Stebbins, 1971). Chromosomes are characterized by their relative size as small, medium and large chromosomes (Singh, 2004).

2.8.1.4. Chromosome number

Chromosome number is usually constant for a particular species (Krawiec, 2003), but may vary between different species. Variations in chromosome number are of two major types; aneuploidy and euploidy (Stebbins, 1971). When chromosome numbers vary in multiples of the complete chromosome set basic to a species, it is termed as euploidy. When the number varies in less than a complete set, it is termed aneuploidy (Sleper and Poehlman, 2006). Differences in chromosome numbers between related taxa are common among plants (De Wet, 1971). In addition to the normal constant chromosome complements, many plant species contain a variable number of chromosomes known as accessory or supernumerary or B-chromosomes (Camacho, 2005). They are genetically dispensable since they do not have any known significant effect on the morphology or physiology of the host plants (Singh, 2004).

2.8.1.4.1. Polyploidy

Polyploidy is an aspect of euploidy in which an individual or cell possesses more than two sets of chromosomes. The term “ploidy” or “ploidy level” refers to the number of complete sets of chromosomes, a set being denoted by an “x” (Ranney, 2006). Therefore, polyploidy is defined

as the heritable condition of possessing more than two complete sets of chromosomes (Ramsey and Schemske, 1998), and it is a prominent and significant force in plant evolution (Adams and Wendel, 2005). The frequency of polyploidy is high in both angiosperms and pteridophytes but it is highly prevalent in pteridophytes than in angiosperms (Otto and Whitton, 2000). In the case of angiosperms, polyploidy is more common in the monocots than the dicots, and within each of these groups the frequency of polyploids varies significantly among families and genera (De Wet, 1971). It has been established that around 60 percent of all plant species are polyploids (Ennos and Sheffield, 2000). Approximately, 70 percent of angiosperms and nearly 90 percent of pteridophytes are polyploids (Sharma and Sen, 2002).

Determination of ploidy level is an essential information in plant breeding and genetics (Cramer, 1999), because ploidy level may determine the number of different alleles that an individual can possess at a given locus (Acquaah, 2007). Polyploidy modifies both the genotype and the phenotype of an organism, generating morphological and physiological changes (Thompson *et al.*, 2004). A high degree of polyploidy in plant species is sometimes associated with apomixes (Singh, 2003b).

2.8.1.4.1.1. Types and origins of polyploidy

Based on its mode of origin, polyploidy can be classified into two distinct types; autopolyploidy and allopolyploidy (Stebbins, 1971; Ramsey and Schemske, 2002). Autopolyploidy is derived from one individual or from crosses within or between populations of a single species, most likely through union of unreduced gametes, whereas allopolyploidy arises from crosses between distinct species forming hybrids and subsequent chromosome doubling (Ramsey and Schemske, 1998). An autopolyploid plant would resemble the original parent, but each homologue occurs in more than two copies per nucleus. An allopolyploid plant would tend to exhibit phenotypes that are intermediate between those of its parental species (Acquaah, 2007). Allopolyploidy has long been considered much more common than autopolyploidy in nature (Otto and Whitton, 2000).

Polyploidy is the most wide-spread and distinctive cytogenetic process which has been considered as a major mechanism of adaptation and speciation in plants (Ramsey and

Schemske, 1998). The high incidence of polyploidy at high latitudes, high altitudes, and glaciated areas may be related to the tendency of harsh environmental conditions to induce the formation of unreduced gametes and results in polyploidy formation (Ramsey and Schemske, 1998). Furthermore, polyploids are capable of tolerating and invading harsh environments better than their diploid counterparts due to changes in physiology resulting from increased cell size (Mable, 2004). Polyploidy can also modify reproductive system, through the breakdown of self-incompatibility (Thompson *et al.*, 2004), and also provides further benefits in areas where breeding systems are compromised in stressful environments (Ranney, 2006). The likelihood of polyploid establishment could be increased even in self-incompatible plants through shifts in flowering time, ecological habitat, or pollinator preference that often accompany polyploidization (Otto and Whitton, 2000).

Compared to their corresponding diploid progenitors, polyploid plants show variation in petal size, leaf size, guard cell size, leaf thickness, stem thickness, stomata density, number and size of germinal pores of the pollen grains and growth rate (Levin, 2002). Inferences about ploidy levels can sometimes be made from the degree of phenotypic manifestations mentioned above. But, definitive ploidy level can be determined by direct count of mitotic and meiotic chromosomes. However, direct count is labor intensive and in many cases not completely reliable if the chromosomes are too small in size and numerous in numbers (Negron-Ortiz, 2007). Alternatively, flow cytometry can be employed to determine ploidy level through determination of nuclear DNA content (Gregory, 2005; Jones, 2007). However, the present study has preferred to use the standard chromosome count due to its requirements for inexpensive cost and crude laboratory equipments to investigate the cytogenetic variability among farmers' varieties of *O. ficus-indica* locally identified in Tigray.

2.8.2. Limitations on the cytogenetic data

As indicated in the preceding pages, chromosome data provides valuable information regarding the taxonomy and phylogenetic relationship of species. However, full information can be obtained only where it is possible to make accurate counts of the chromosome number and describe the chromosome morphology. Unfortunately, the chromosome of a number of

plant species are either difficult to count accurately or describe their morphology or may be difficult both for chromosome count and morphological description.

In plants, the chromosome number varies from 2n = 4 in *Haplopappus gracillis* (Compositae) to 2n = >1200 in some pteridophytes, and the size ranges from less than a micrometer in *Drosera* (Stebbins, 1971) to about 30 µm in *Trillium* (Gupta, 2008). Generally, among plants, the monocotyledonous species have large chromosomes which can be easily counted and accurately described morphologically such as, *Aloe* species, (Bradham and Doherty, 1998; Alemayehu Mekuria, 2007; Tezera Temesgen, 2008), *Kniphofia* species (Fikadu Gadissa, 2009), and *Fritillaria* species (Bennett and Leitch, 2005b). Here again, even though it is easy to describe the karyotype for individual species, in some cases like in the genus *Aloe,* the karyotypes are so similar across the genus and sometimes even across related genera that it is not possible to distinguish species on the basis of their karyotypes (Bradham and Doherty, 1998). In such cases, relatedness but not differences can be inferred from the chromosome data.

In some other plant species, such as *Myriophyllum spicatum*, 2n = 14, and *Selaginella kraussiana,* 2n = 40, the chromosome number is small enough to count, but their size is too small to describe their morphology (Bennett and Leitch, 2005b). There are also other plant species such as *Phytolacca dodecandra*, 2n ≈ 140 (Kifle Dagne, personal communication), *Equisetum variegatum,* 2n ≈ 216 (Bennett and Leitch, 2005b), *Ophioglossum petiolatum*, 2n ≈ 960 (Obermayer *et al.,* 2002), and *Ophioglossum Variegatum,* 2n ≈ 1260 (Stebbins, 1971), the chromosomes are not only too small in size, but are also many in numbers, where accurate counting is difficult, let alone, making morphological description. Chromosomes of some plant species like *Luzula purpurea* are also holocentric that lack centromeric constriction (Stebbins, 1971). Thus, it is evident that the various cytological features discussed in the preceding pages may not be appropriate for karyotyping and morphological characterization of chromosomes of many plant species, particularly for those species that have numerous and small-sized chromosomes.

2.9. Objectives of the Study

2.9.1. General objective

The general objective of the present study is to generate the cytogenetical information on farmers' varieties of *Opuntia ficus-indica* locally identified in Tigray, Northern Ethiopia.

2.9.1.1. Specific objectives

The present study is intended to:

i. determine chromosome number of the different local farmers' varieties.
ii. establish ploidy levels of the different local farmers'varieties of *O. ficus-indica*.
iii. compare chromosome counts of these local farmers' varieties with that of other specimens of *O. ficus-indica* and *O. cylinderica* collected from some other localities.

3. MATERIALS AND METHODS

3.1. Plant materials

Cladode cuttings of thirty four different local farmers' varieties of *O. ficus-indica* were obtained from a Germplasm Nursery Orchard established by the Tigray Agricultural Research Institute at Mekelle town. The local farmers' varieties that were kindly provided by the Research Institute include twelve varieties from Woreda "Mekhoni" (Table 1), nine varieties from Woreda "Ganta-afeshum" (Table 2), and thirteen varieties from Woreda "Erob" (Table 3). Two additional *O. ficus-indica* plants, each from Debre-zeyit and Dire Dawa, and one out-group specimen of *O. cylinderica* from Addis Ababa were included in the study for comparison. Individual cuttings from each sample plant were planted in separate pots, with all provisions necessary for rooting of the cuttings in a greenhouse at 'Arat-Kilo' Campus of Science Faculty, Addis Ababa University (Figure 5).

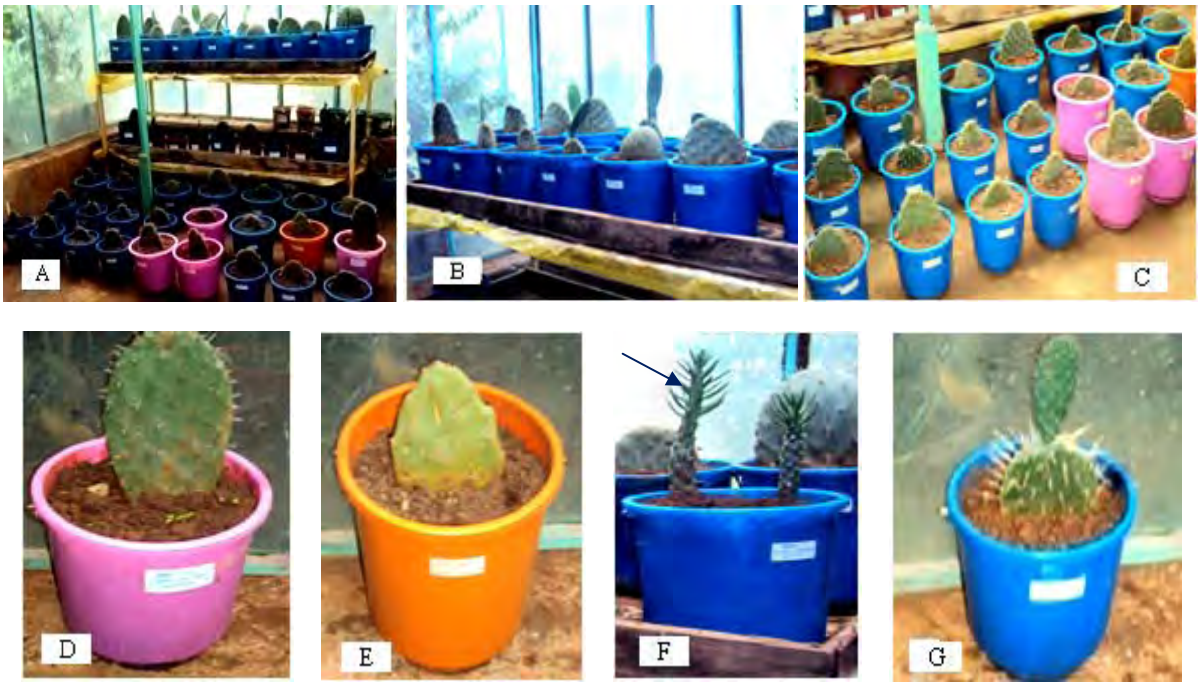

Figure: 5. *Opuntia ficus-indica* and *O. cylinderica* plants potted in a greenhouse. A) All sample plants used for the study, B) Varieties from Woreda"Mekhoni", C) Varieties from both Woreda "Ganta-afeshum" and "Erob, D) *O. ficus-indica* plant from Debre-zeyit, E) *O. ficus-indica* plant from Dire Dawa, F) *O. cylinderica* plant (arrowed) from AAU, and G) A sample plant with a young cladode grown from the parent cladode.

3.2. Root collection

For mitotic chromosome preparation, fresh root tips were collected between 8:00 am and 9:00 am. This is a little modification on the root harvesting time, 7:00 am to 9:00 am, used by Spencer (1955), and Cota and Philbrick (1994) for somatic metaphase chromosome preparation of species in the family Cactaceae.

3.3. Pretreatment and maceration

The collected root-tips were washed in tap water and subjected to appropriate pre-treatment as described by Kifle Dagne and Heneen (1992), either kept in ice-cold water for about 24 hours or treated in 0.002 M 8-hydroxyquinoline for about 3-4 hrs or in 0.05% colchicines for 1-2 hrs at room temperature. However, the ice-cold water pretreatment method was found more preferable in arresting chromosomes of sample plants of the study at their maximum state of condensation. The pretreatment was followed by fixation of the root tips in 3:1 absolute alcohol to glacial acetic acid (v/v) for about 24 hrs at 4 ^{0}C, and the root tips were stored in 70% alcohol after fixation until they are needed for chromosome preparation. In order to macerate the root tips preserved in 70% ethanol, the combination of hydrolysis in hydrochloric acid followed by enzyme maceration were used. Root-tips were thoroughly rinsed in distilled water and hydrolyzed in 1N HCl aqueous for about 10 minutes at 60 ^{0}C, then thoroughly rinsed in distilled water, which was followed by maceration in 4% cellulase and 4% pectinase (pH 4 - 4.5) for about an hour in a water bath adjusted at 37 ^{0}C (Kifle Dagne and Heneen, 1992; Kifle Dagne, 1995).

3.4. Air-dry chromosome slide preparation

Air-dry chromosome preparation was made according to the method used by Kifle Dagne and Heneen (1992). After rinsing with distilled water, the macerated root-tips were placed on a clean glass slide using a Pasteur pipette. The water surrounding the root-tips was carefully removed using a filter paper, and the root tips were mashed in a drop or few drops of fresh fixative (3:1 ethanol to acetic acid) using a mounted needle having a flat end. A strong air

blow was applied on the slide to spread the individual cells, and the slide was allowed to air dry at room temperature and stored in a slide box until it is needed for staining.

3.5. Staining

The air-dried slides were stained through the procedure used by Kifle Dagne (1995), in 2.5% Giemsa stain in Sorensen's phosphate buffer (pH 6.8) for about an hour, and then rinsed in distilled water and allowed to air dry. Finally, the slides were mounted permanently using cover slips in DePeX (Kifle Dagne and Heneen, 1992).

3.6. Chromosome counting

The permanent mounts were observed under light microscope, and cells with better chromosome spreads were photographed with a camera-fitted microscope using X100 objective. Chromosome numbers were generally determined for each sample plant through individual chromosome count made on more than one intact cell, except a few sample plants for which only a single representative cell was counted.

The photomicrographs of mitotic chromosome spreads of the sample plants with better chromosome spreads and condensation were adjusted with the MBF ImageJ software in order to improve the contrast of the photomicrographs and enhance the distinguishable view of individual chromosomes in a cell.

4. RESULTS

All the local farmers' varieties of *O. ficus-indica* that were obtained from Tigray Agricultural Research Institute (TARI) and specimens collected from some other localities of Ethiopia were included in the study, and chromosome slides were prepared for all sample plants. However, cells with countable chromosome preparation were not obtained for those of thirteen local farmers' varieties of *O. ficus-indica.* Only those twenty-one local farmers' varieties and those three sample plant specimens of some other localities for which countable chromosome spreads were obtained are presented as representatives here under. Chromosome numbers in the present study were generally determined from cells, in which individual chromosomes were clearly distinguishable, and countable with ease. These plant specimens are also summarized in Tables 4-6, and photomicrographs of the representative plant specimens are presented in Figures 6-9.

However, it should be noted that individual photomicrograph may or may not depict the maximum chromosome count recorded for the representative plant specimens summarized in the Tables 4-6. Just, they have preferred to present in Figures 6-9 as representative photomicrographs because of their better chromosome spreads.

4.1. Chromosome counts for local farmers' varieties of *O. ficus-india* from Woreda "Mekhoni"

The present study has included twelve different local farmers' varieties of *O. ficus-indica* from Woreda "Mekhoni" for somatic chromosome counts. However, plausible chromosome counts were found only for nine of the local farmers' varieties of this locality (Table 4). The result for the remaining three local farmers' varieties (Tsaeda Aona, Meskel Gedaho and Chewchawe) could not be included in the table because cells with countable chromosome spreads were not obtained for them, although it was possible to estimate roughly that they are polyploids, presumably of same ploidy level as those for which counts are available.

Table: 4. Summary of chromosome counts for local farmers' varieties of *O. ficus-indica* of Woreda "Mekhoni".

No.	Local farmers' variety name	Number of potted plant per variety	Number of metaphase plates counted	Maximum Chromosome count recorded	Possible somatic * chromosome number (2n)
1.	Megal Hailu	01	02	88	88
2.	Shenkor	01	03	85	88
3.	Berbere Hailesilassie	01	03	86	88
4.	Limo	01	01	86	88
5.	Tesemsema	01	07	87	88
6.	Mot Kolea	01	04	88	88
7.	AwKulukual Bahri	01	01	88	88
8.	Cheguar	01	05	87	88
9.	Fukur Rosso-Adem	01	03	86	88

* The possible somatic chromosome number given in the table is based on the established basic number of the species in the family Cactaceae, $x = 11$ (Ross, 1981), with a ploidy level of 8.

The light photomicrographs presented below in Figure 6 show representative somatic chromosome spreads of local farmers' varieties of *O. ficus-indica* identified in Woreda "Mekhoni".

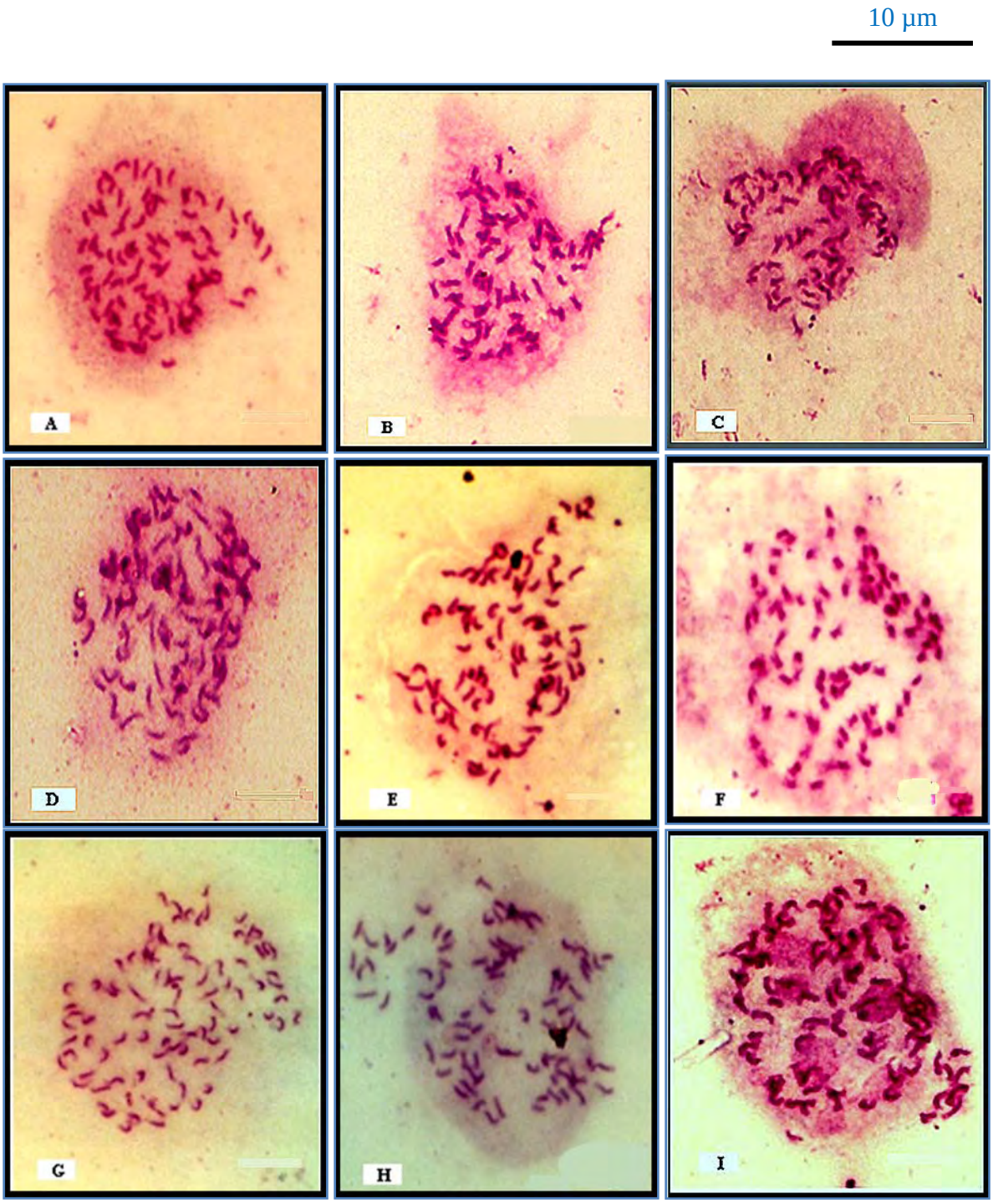

Figure: 6. Somatic chromosome spreads of local farmers' varieties of *O. ficus-indica* of Woreda "Mekhoni". A) Megal Hailu, B) Shenkor, C) Berbere Hailesilassie, D) Limo, E) Tesemsama, F) Mot-kolea, G) Awkulkual Bahri, H) Cheguar**,** and I) Fukur Rosso-adem.

4.2. Chromosome counts for local farmers' varieties of *O. ficus-indica* from Woreda "Ganta-afeshum"

In the present study, Woreda "Ganta-afeshum" was represented with nine locally identified farmers' varieties of *O. ficus-indica* (Table 2). Unfortunately, countable chromosome spreads were found for only three of the local farmers' varieties (Table 5). The remaining six local farmers' varieties do not have chromosome counts due to poor chromosome spreads.

Table: 5. Summary of chromosome counts for local farmers' varieties of *O. ficus-indica* of Woreda "Ganta-afeshum".

No.	Local farmers' variety name	Number of potted plant per variety	Number of metaphase plates counted	Maximum Chromosome count recorded	Possible somatic * chromosome number (2n)
1.	Koremele	01	03	84	88
2.	Lemats Beles	01	01	86	88
3.	Machuch	01	01	87	88

* The possible somatic chromosome number given in the table is based on the established basic number of the species in the family Cactaceae, $x = 11$ (Ross, 1981), with a ploidy level of 8.

Figure 7 presents somatic chromosome spreads of three local farmers' varieties of *O. ficus-indica* identified in Woreda "Ganta-afeshum". The spreads showed that those local farmers' varieties have the same ploidy level, with an octoploid cytotype and somatic chromosome number of $2n = 8x = 88$.

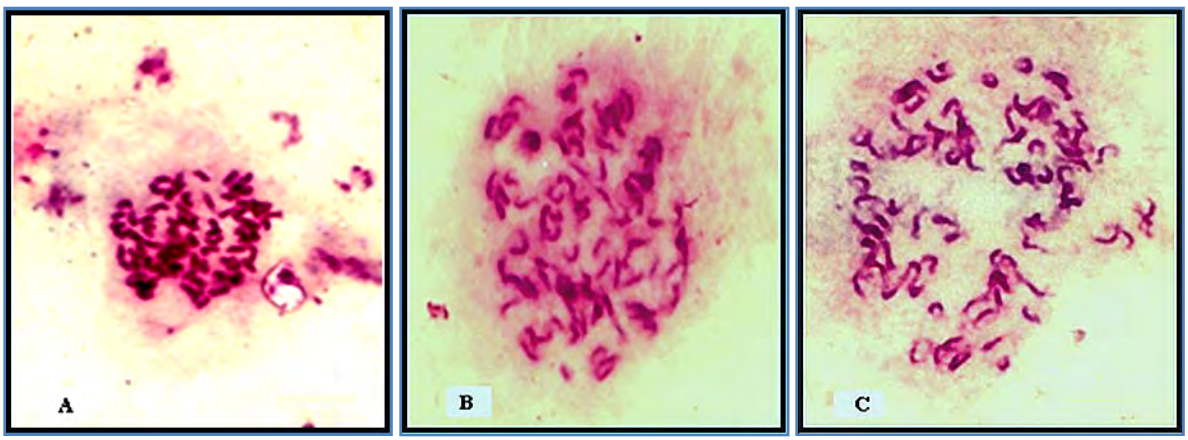

Figure: 7. Somatic chromosome spreads of local farmers' varieties of *O. ficus-indica* of Woreda "Ganta-afeshum". A) Koremele, B) Lemats Beles, and C) Machuch.

4.3. Chromosome counts for local farmers' varieties of *O. ficus-indica* from Woreda "Erob"

Woreda "Erob" was also represented in the present study, with a total of thirteen local farmers' varieties of *O. ficus-indica* (Table 3), but chromosome counts are available for only those nine local farmers' varieties (Table 6). Counts for the remaining four local farmers' varieties (Adomulhata, Gerwanlyele, Suluhna and Ameudega Adobelesa) could not be obtained because of poor chromosome spreads.

Table: 6. Summary of chromosome counts for local farmers' varieties of *O. ficus-indica* of Woreda "Erob".

No.	Local farmers' variety name	Number of potted plant per variety	Number of metaphase plates counted	Maximum Chromosome count recorded	Possible somatic * chromosome number (2n)
1.	Gerao	01	04	86	88
2.	Nahrisa	01	02	88	88
3.	Kalamile	01	02	86	88
4.	Geleweiti	01	02	88	88
5.	Hiraydaglayele	01	01	83	88
6.	Neitsi	01	05	86	88
7.	Orgufa	01	03	83	88
8.	Hawawisa	01	01	85	88
9.	Asa Kurkura	01	02	86	88

* The possible somatic chromosome number given in the table is based on the established basic number of the species in the family Cactaceae, $x = 11$ (Ross, 1981), with a ploidy level of 8.

Photomicrographic representations of somatic chromosome spreads are given below for the representative local farmers' varieties of *O. ficus-indica* of Woreda "Erob" (Figure 8). These photomicrographs showed that the local farmers' varieties of Woreda "Erob" examined in the present study were polyploids, with ploidy level of eight similar to that of the local farmers' varieties of *O. ficus-indica* collected from other Woredas of Tigray (Figures 6-7).

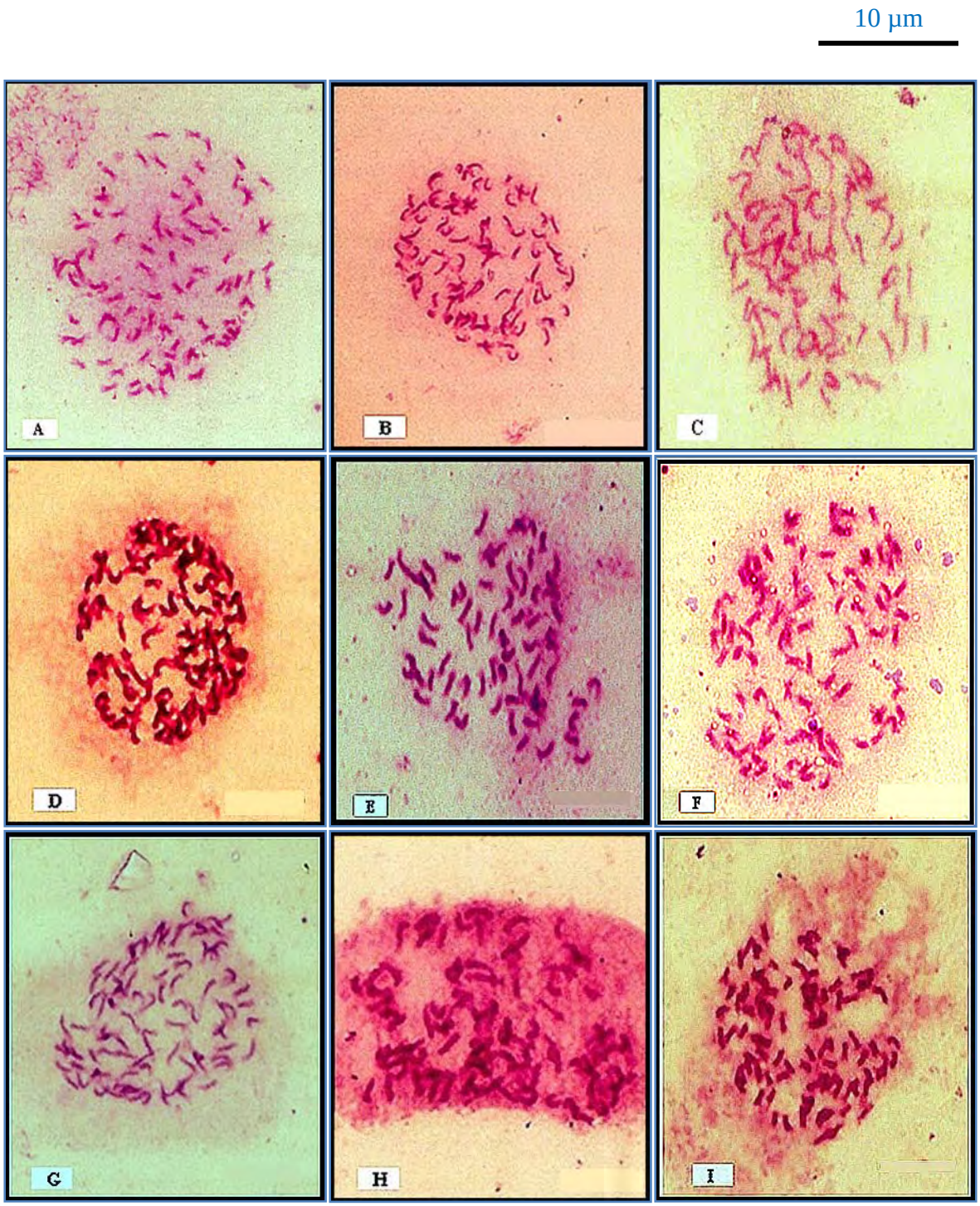

Figure: 8. Somatic chromosome spreads of local farmers' varieties of *O. ficus-indica* of Woreda "Erob". A) Gerao, B) Nahrisa, C) Kalamile, D) Geleweiti, E) Hiraydaglayele, F) Netsi, G) Orgufa, H) Hawawisa, and I) Asakurkura.

4.4. Chromosome counts for *Opuntia* species from some other localities of Ethiopia

Chromosome counts were also conducted on other three *Opuntia* plants collected from some other localities of Ethiopia so as to compare their chromosome counts with that of local farmers' varieties of *O. ficus-indica* collected from Tigray. These plant collections include two plant specimens of *O. ficus-indica*, each from Debre-zeyit and Dire Dawa, and one out-group plant specimen of *O. cylinderica* from Addis Ababa, Campus of Science Faculty. Their chromosome counts are also summarized in Table 7.

Table: 7. Summary of chromosome counts for *O. ficus-indica* and *O. cylinderica* plants collected from some other localities of Ethiopia.

No.	Plant specimen	Number of potted plant per variety	Number of metaphase plates counted	Maximum Chromosome count recorded	Possible somatic * chromosome number (2n)
1.	*O. ficus-indica* [1]	01	03	86	88
2.	*O. ficus-indica* [2]	01	01	85	88
3.	*O. cylinderica*	01	04	118	121

* The possible somatic chromosome number given in the table is based on the established basic number of the species in the family Cactaceae, $x = 11$ (Ross, 1981), with a ploidy level of *8* for *O. ficus-indica* and ploidy level of *11* for *O. cylinderica*.

The first two plant specimens of *O. ficus-indica* in Table 7 are distinguished from each other with superscript numbers, 1 and 2, to represent their collection sites, each from Debre-zeyit and Dire Dawa, respectively.

The following somatic chromosome plates in Figure 9 are presented for comparison of the local farmers' varieties of *O. ficus-indica* collected from Tigray with other two *O. ficus-indica* plants collected from Debre-zeyit and Dire Dawa, and one *O. cylinderica* plant collected from Addis Ababa.

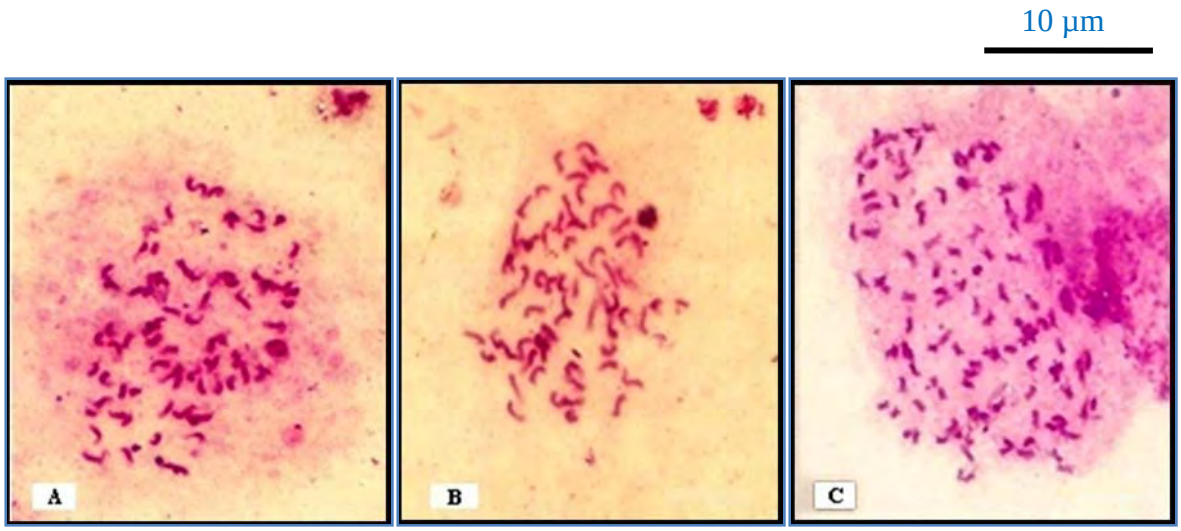

Figure: 9. Somatic chromosome spreads of *O. ficus-indica* and *O. cylinderica* plants from some other localities of Ethiopia. A) *O. ficus-indica* from Debre-zeyit, B) *O. ficus-indica* from Dire Dawa, and C) *O. cylinderica* from AAU, Campus of Science Faculty.

The *O. ficus-indica* plants that were collected both from the shore of lake "Hora" (in Debre-zeyit) and Dire Dawa were found octoploids with a somatic chromosome number of *2n = 8x = 88*. However, the *O. cylinderica* plant that was collected from "Arat-kilo" Campus of Addis Ababa University showed a ploidy level different from that of the *O. ficus-indica* specimens observed in the study. This *O. cylinderica* plant was also found polyploid with a somatic chromosome number of *2n = 11x = 121*.

5. DISCUSSION

Chromosome counting is a relatively easy task which produces reliable and highly reproducible data, unless the chromosome number of the specimen is very high (Guerra, 2008). The findings of the present study have shown that the chromosomes of *Opuntia ficus-indica* are very small in size and numerous in numbers. Therefore, it is a challenging and tedious task to obtain the precise chromosome counts for all the local farmers' varieties of *O. ficus-indica* included in the study. According to Guerra (2008), determinations of chromosome counts for specimens with larger chromosome numbers are often less precise, although in such cases, approximate count may be enough for cytotaxonomic purposes. Therefore, an approximate chromosome count nearest to the possible somatic chromosome number was recorded in the present study through standard chromosome counts based on maximum counts. In most cases, more than one intact cell was counted for each sample plant, except for a few of the sample plants for which a count was obtained only for a single cell.

The fact that, the difficulty to make an accurate chromosome count for *O. ficus-indica* plants, the possible chromosome number was inferred from the proximity of the count to *8x* ploidy level based on the base number previously reported for the species in the family Cactaceae. In the first place, the chromosomes are too many in numbers and too small in sizes which contribute to the imprecision of the chromosome count. In the second place, chromosomes are clumped together in most of the cells undergoing mitosis, so it is difficult to count them precisely. Even in those very rare cases where chromosomes are well spread and countable, the cell might have lost some of its chromosomes in the process of slide preparation and so one cannot always get complete number of chromosomes. In spite of these difficulties, however, there is rare possibility of getting a cell with well spread and complete or nearly complete chromosome number.

Several cytogenetic studies established that the species of family Cactaceae have a base number of $x = 11$ (Spencer, 1955; Ross, 1981). Compared to the base number, the chromosome counts in the present study indicated that these traditionally identified farmers' varieties of *O. ficus-indica* are polyploids, and they all belong to a single ploidy level that is an octoploid ($2n = 8x = 88$, where $x = 11$). The counts confirmed that all of the local farmers'

varieties of *O. ficus-indica* collected from Tigray as well as *O. ficus-indica* plants colleced from Debre-zeyit and Dire Dawa are octoploids with basic chromosome number of $x = 11$. The findings of the present study are also in line with Pimienta (1995), whose review indicated polyploidy as a common phenomenon within the genus *Opuntia*.

Results of the present study have revealed that there was no variation in ploidy level among local farmers' varieties of *O. ficus-indica* of Tigray as well as *O. ficus-indica* plant specimens included in the study from some other localities of Ethiopia. Even though, people assumed that some of the differences in fruit characteristics and cladode morphology that exist among wild as well as cultivated populations of *O. ficus-indica* are likely to be due to differences in ploidy levels (Mondragon, 2001a), the present findings have revealed that the different local farmers' varieties of *O. ficus-indica,* with different fruit characteristics and cladode morphology, belong to the same ploidy level. Thus, unlike the assumptions of some people, the variations in their fruit characteristics and cladode morphology cannot be attributed to the differences in ploidy level, since the present study has shown that all the local farmers' varieties investigated have the same ploidy level regardless of their fruit characteristics and cladode morphology.

Cactus plants were previously reported as they have shown high phenotypic plasticity that may result in phenotypic variability (Mondragon, 2001a). Similarly, some aspects of the variability in plant morphology and fruit characteristics may have been resulted by the environmental factors exerting phenotypic plasticity.

Boyele and Anderson (2002) described that the genetic variability in cultivated cacti is limited due to narrow genetic bases of the cultivars. This low genetic variability may also apply to the *O. ficus-indica* populations of Ethiopia because of the fact that they are introductions from elsewhere (Vigueras and Mondragon, 2005), and they may suffer from the founder effect. However, in spite of the assumed narrow genetic base, some of the variability in the *O. ficus-indica* populations should definitely be due to genetic cases, which should be studied and exploited for the improvement of the plant.

According to Tesfay Belay *et al.* (2009), of the local varieties of *O. ficus-indica* of Woreda "Mekhoni", local farmers' varieties "Shenkor" and "Tesemsama" have better fruit tastes than

others. However, those varieties possessing sweet fruit tastes have the same ploidy level like that of other local farmers' varieties of the same locality. Furthermore, local farmers' variety "Limo" represents the spineless variety in Woreda "Mekhoni", but all the other varieties of this locality are spiny. Once more, both those spiny and spineless varieties have no variation in their ploidy level, and the same is true for the other local farmers' varieties of Woreda "Ganta-afeshum" and Woreda "Erob". Therefore, the origin for their qualitative and quantitative variations that have been observed in the study of Tesfay Belay *et al.* (2009) may be linked to the existence of allelic variation at loci governing those traits. However, the degree of allelic variability, their mode of inheritance and action may be explained through proper crossing experiments.

Due to the fact that, polyploids could either be 'auto-' or 'allo- ' polyploids (Ramsey and Schemske, 2002), it is difficult to suppose unambiguously from the present study, as to which of these occurs in *Opuntia* species. Because meiotic chromosome study was not included in the present work due to the long time the plant takes to attain its flowering stage after its vegetative propagation.

As shown by Ross (1981) as well as the present study, cactus chromosomes have few distinctive morphological characters during mitosis owing to their small size besides being large in number. These features make it difficult to construct the karyotype for any of the local farmers' varieties of *O. ficus-indica* collected from Tigray as well as for those plant specimens collected from some other localities of Ethiopia.

Since plants of the same species with lower ploidy levels are more primitive than the higher ones of their descendants (Sharma and Sen, 2002), the local farmers' varieties of *O. ficus-indica* examined in the present study can be grouped with those cultivated types due to their higher ploidy levels. This may also give a clue as these *O. ficus-indica* examined in the study were derived from cultivated varieties found somewhere, and an increase in ploidy level either with or without hybridization often associated with speciation and the origin of novel adaptation (Levin, 2002). Polyploid species are often adapted to diverse ecological conditions by having a broader spectrum of tolerance (Levin, 2002), thus *O. ficus-indica* plants have an evolutionary potential to march into new habitats.

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The present study has determined the chromosome numbers and ploidy level of the different local farmers' varieties of *O. ficus-indica* growing in Tigray, Northern Ethiopia. Accordingly, it was found that all the local farmers' varieties included in the study, particularly those for which chromosome counts were obtained, are octoploids with a somatic chromosome number of *2n = 8x = 88*.

Furthermore, the present study has also shown that the variations in fruit characteristics and cladode morphology that exist among the various local farmers' varieties of *O. ficus-indica* plants are determined independent of the ploidy level since plants belonging to the same ploidy level vary with regard to these characteristics. Further, this implies that the variations in fruit characteristics and cladode morphology have genetic bases other than the ploidy level. However, the present study could neither determine the polyploidy nature nor establish the karyotypic description, due to the prolonged time the plant takes to flower in the former case and the latter one is due to numerous and small-sized chromosomes of *O. ficus-indica*.

Although it is difficult to draw strong conclusion simply from comparisons of chromosome counts, the present results may lead to a speculation that all the locally identified farmers' varieties of *O. ficus-indica* growing in Tigray, and the *O. ficus-indica* collections of Debre-zeyit and Dire Dawa may have originated from the same sources, possibly descended from same plants originally introduced into Ethiopia, unless repeated introduction at different times in different parts of the country are assumed. However, the sample plant of *O. cylinderica* was found to be different from sample plants of *O. ficus-indica* in morphology and chromosome number.

6.2. Recommendations

Generally, *Opuntia ficus-indica* has been reported to have actual and potential economic importance, specifically for those arid and semi-arid regions of the world. Therefore, based on the findings of the present study, the following recommendations can be made:

- Almost all the plant materials used in the present study were collected from three Woredas of Tigray Region. Therefore, further chromosome study is recommended in covering wide populations of *O. ficus-indica* collected from different parts of Ethiopia.

- As the present study could not determine the nature of polyploidy in *O. ficus-indica*, it is recommended to determine whether the observed polyploidy is autopolyploid or allopolyploid, using appropriate methods such as meiotic chromosome analysis or molecular approaches. The distinction between the two major types of polyploidy is important for future breeding of *O. ficus-indica* since it notifies whether the plant has disomoc or polysomic mode of inheritance.

- Determination of chromosome numbers through microscopic count is tedious and time consuming, particularly when one is dealing with large number of plants, having chromosomes very small in size and large in number like that of *O. ficus-indica*. Thus, it is recommendable to use flow cytometry method in determining the ploidy level of such plants.

- In Ethiopia, the cactus plant is predominantly propagated by vegetative means that does not allow genetic recombination. So, it is recommended to raise populations from seeds and evaluate for their genetic variability, which would lead to the improvement of the cactus plant through selection of desirable recombinants.

- Polyploidy is usually associated with apomixis. Thus, the nature of seed formation in *O. ficus-indica* plants should be examined through further studies to determine whether the plant produces apomictic or sexual seeds, because this will have a great bearing on the option of breeding strategy to be followed in the future.

- Molecular markers, such as ISSR and AFLP are ideal techniques to reveal the genetic variability in populations. Therefore, utilization of molecular markers is recommended to assess the genetic variability within and among *O. ficus-indica* populations in Ethiopia.

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