UNIVERSITY OF ZULULAND

GENETIC IMPROVEMENT OF SELECTED INDIGENOUS CUCURBITACEAE SPECIES IMPORTANT FOR FOOD AND MEDICINAL PURPOSES IN KWAZULU-NATAL, SOUTH AFRICA

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by

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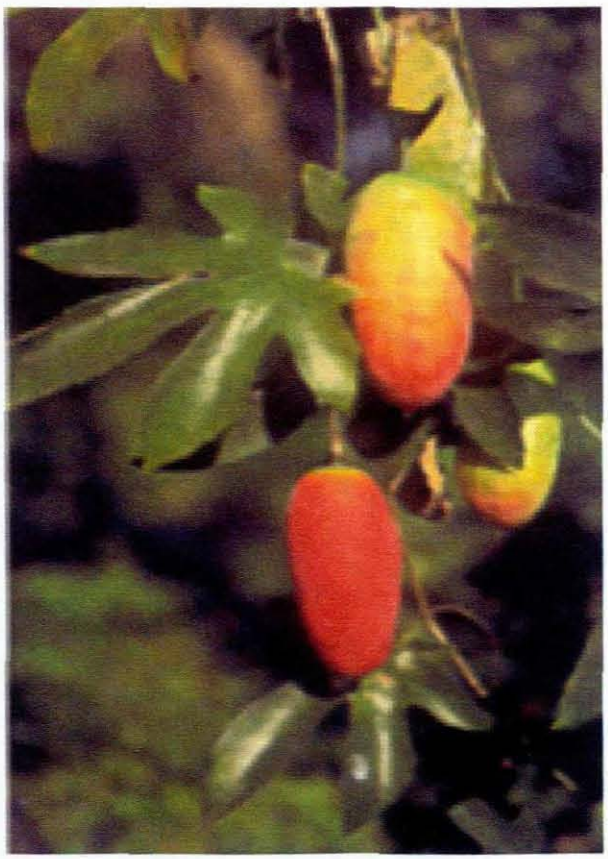
Coccinia palmata



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PREFACE

The work described in this dissertation was carried out in the Department of Botany at the University of Zululand, KwaDlangezwa and at the Agricultural Research Council – Institute of Tropical and Subtropical Crops (ARC-ITSC) at Nelspruit, Mpumalanga Province, under the supervision of Prof. D.P. Ferreira.

These studies have not otherwise been submitted in any form for any degree or diploma at any University. Where use has been made of work of others, it is duly acknowledged in the text.

Ntuli

Nontuthuko Rosemary Ntuli

I certify that the above statement is correct.

Ferreira

Professor D.P. Ferreira

ABSTRACT

Coccinia palmata (Cogn.) and *Lagenaria sphaerica* (Sond.) Naud. are indigenous Cucurbitaceae species widely distributed in pantropical to tropical regions of Southern Africa. They are widely used for food and medicinal purposes. In nature these species exist as diploids, with their basic haploid (n) chromosome numbers being 12 and 11 for *C. palmata* and *L. sphaerica* respectively. However, in higher plants nuclear DNA endoreduplication often occurs during their growth and differentiation which causes polysomaty in their tissues.

For both *C. palmata* and *L. sphaerica*, the optimum germination conditions were 25°C under light. Growth was either in the nursery (in pots/bags) or in the field (with and without bags). Colchicine treatment of seeds before and after incubation for germination was used to induce polyploidy in both of these Cucurbitaceae species. The effectiveness of colchicine in inducing polyploidy (genetic improvement) in the roots and shoots of both species was established. In both species treated roots had higher tetraploid and octoploid ratios than leaves. Colchicine treatment lowered the germinability (germination percentage), induced dwarfism, enhanced stem branching, reduced leaf area and number of stomata, and increased guard cell length and leaf chlorophyll content in both species. In *C. palmata*, colchicine treatment increased root fresh and dry weight but reduced shoot fresh and dry weight. However, colchicine treatment inhibited female plants' flowering and therefore fruiting in both species.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

IMS:	Industrial Methylated Spirits
SEM:	Scanning electron microscope
F_1:	Filial one (first generation from the parent plants especially after crossing)
EMS:	Ethyl methanesulphonate
MMS:	Methyl methanesuphonate
IAEA:	International Agricultural Exchange Association
DNA:	Deoxyribonucleic acid
RH:	Relative humidity
ISTA:	International Seed Testing Association
PAT:	Polar auxin transport
P_r:	Phytochrome red
P_{fr}:	Phytochrome far red
BRs:	Brassinosteroids
GA:	Gibberellic acid
Gas:	Gibberellins
IAA:	Indole-3-acetic acid
ILVs:	Indigenous Leafy Vegetables
TLVs:	Traditional Leafy Vegetables
ZULU:	University of Zululand herbarium
KNH:	KwaZulu-Natal Herbarium
OD:	Optical Density
DMSO:	Dimethyl sulphoxide / Dimethyl sulfoxide
Chla:	Chlorophyll a
Chlb:	Chlorophyll b
tot Chl:	Total Chlorophyll
g/l:	grams per liter
mg/cm^3:	milligrams per cubic centimeters or per milliliter
Inc. days:	Incubation period in days

T°C:	Temperature in degrees Celsius
Sod. hyp.:	Sodium hypochlorite
Ster.:	Sterilization procedure
Germ.:	Germination
SD:	Standard deviation
2n:	diploid
3n:	triploid
4n:	tetraploid
5n:	pentaploid
6n:	hexaploid
7n:	heptoploid
8n:	octoploid

LIST OF APPENDIXES

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Above all, I worship GOD Almighty in Jesus' Name for His grace and mercy which brought me through, especially His Spirit of Wisdom, Knowledge and Understanding which has helped me throughout this study.

Lagenaria sphaerica

CHAPTER ONE

Introduction

The family Cucurbitaceae consists of about 119 genera and 850 species (Andres 2004, Berrie 1977, Dyer 1975, Jeffrey 1978, Pooley 1998). Of these, 18 genera occur in southern Africa (Pooley 1998). Plants in this family mostly range from tropical to sub-tropical regions where they grow in grasslands, forest margins as well as in the forests but some species are reported to occur in deserts (Moss 1994). As a result, they grow in the range from full sunlight through to full shade conditions (Andres 2004).

The family Cucurbitaceae is herbaceous and is commonly called the Cucumber/ Pumpkin/ Gourd family. Stems are prostrate, trailing or climbing with spirally coiled tendrils. Leaves are simple or lobed. Male and female flowers occur on the same or on separate plants. Female flowers are fleshy, 5 lobed with an inferior ovary and 2-3 lobed stigma. Fruits are usually fleshy and seeds are flattened. Pumpkins, squashes, cucumbers, watermelons and ornamental gourds and luffas, to name a few, are important food plants that belong to the family Cucurbitaceae (Pooley 1998).

In nature the plants normally occur as diploids (having two sets of chromosomes). If the formation of spindles during metaphase of mitosis is inhibited, the set of chromosomes is then doubled and becomes four. A plant with four sets of chromosomes is referred to as a tetraploid plant. If the same process is allowed to occur with a tetraploid plant, the result will be eight sets of chromosomes which is called an octoploid. Thus every plant with more than two sets of chromosomes is referred to as a polyploid. An alkaloid called colchicine, derived from *Colchicum automnale* L., has been used universally to induce polyploidy (chromosome doubling) in plants (Broertjies and van Harten 1988).

Chromosome doubling (polyploidisation) usually results in an increase in cell volume and therefore of plant parts. Polyploids are expected to have at least one of the following characteristics: larger tuber, rhizome or root size; enhanced fruit size; enhanced flower size and / or colour intensity; improved drought tolerance; increased biomass; improved photosynthetic capacity; larger and/or thicker leaves; dwarfism and increased secondary metabolite production such as medicinal compounds (Tilney-Bassett 1986, Takamura and Mayajima 1996, Hannweg and Vos 2001, Sliwinska and Lukaszewska 2005, Snyder *et al.* 1985, Martelotto *et al.* 2005).

Chromosome doubling affects the nuclear size, cell size and morphology of the plants after induction. The selection and propagation of polyploid plants requires a more detailed analysis in order to determine the precise nature of the end-product (Tilney-Bassett 1986). A strong association between polyploidy and possible structural deformities and morphological changes is assumed.

Identification of polyploid tissue is based on nuclear size, cell size and where possible, chromosome counts. One type of tissue which is very easy to examine is the leaf epidermis. Microscopic examination of the lower epidermis of thorn apple, cranberry and potato leaves reveals that stomata of the polyploids are significantly larger than those of corresponding diploid epidermis (Tilney-Bassett 1986). Some researchers have confirmed that chromosome doubling also diminishes the stomatal frequency of a leaf and enhances the stomatal guard cell length (Beck *et al.* 2003).

Regardless of the advantages of chromosome doubling, disadvantages such as reduction in resistance to drought and cold, lower fertility and yield have been reported in some plant species (Tilney-Bassett 1986). Further breeding and selection at the polyploid level can overcome these disadvantages.

Most of the indigenous Cucurbitaceae species are grouped with traditionally leafy vegetables (TLVs) that have high nutrient contents but are now rejected by many communities, especially in urban areas, which cause a lack of knowledge about their propagation, improvement and commercialization (Chigumira Ngwerume and Mvere 1999).

Indigenous Cucurbitaceae species are used medicinally as a diuretic and commercially for cosmetic purposes (Ahmad and Sharma 2002). Exotic species are mainly utilized in the commercial markets and pharmaceutical industries. The improvement of indigenous species and their introduction as commercial agricultural crops and to the phamarceutical industries will benefit the local economy. Proper, reliable and cost-effective propagation and cultivation methods for these plants will therefore be of crucial importance.

This research has been conducted to establish whether polyploidy induction can be effectively induced in selected indigenous Cucurbitaceae species occurring in Zululand, KwaZulu-Natal, South Africa, and to determine the effect of polyploidy on growth and development of two species, namely *Coccinia palmata* and *Lagenaria sphaerica*. To achieve this, the following objectives were identified:

- To collect, prepare and identify specimens of the species of the Cucurbitaceae family occurring in the studied area.
- To determine the species' usage by local communities through questionnaires.
- To identify species important for food and medicinal purposes.
- To study germination and polyploidy induction using collected seeds of the identified species.
- To determine the effect of chromosome doubling on seed germination, plant growth and yield.
- To determine the suitable light conditions for growth of the polyploid plants.
- To investigate changes in the morphological features of polyploid plants.

- To determine the polyploid yield of plant tissues (root and leaves) originating from colchicine-treated germinating seeds through flow cytometry assays.
- To determine chlorophyll production as influenced by polyploidy.

CHAPTER TWO

Literature Review

2.1 Collection, classification and identification

2.1.1 Collecting purposes and herbarium collection

Plant specimens that are stored in herbaria usually arise from collection according to a specific collecting purpose (Victor *et al.* 2004). Collection may be for taxonomic (research or specialized projects), identification and floristic purposes. The taxonomic and identification collecting types will be discussed as they were adapted for this research.

2.1.1.1 Collecting purposes

Taxonomic collecting is usually part of a research project, concentrating on specific groups and is usually confined to plants within a specific family, genus or species. Voucher specimens for cytological and anatomical projects also fall into this category. The prepared voucher specimen, of a good quality, is deposited in a reputable herbarium to ensure that the identity of the subject of the research can always be verified (Fish 1999, Victor *et al.* 2004). This collecting type enables the documentation of the approximate species distribution within the targeted area.

Identification collecting involves the collecting of plants or plant species whereby a name is needed to carry out a specific project, for example, the food source of insects, weeds for herbicide trials, possible species for introduction into gardens, or environmental impact studies (Fish 1999, Victor *et al.* 2004).

2.1.1.2 Herbarium specimen collection

The Cucurbitaceae family has male and female flowers occurring on the same or separate plants. Plant collection, therefore, has to take this into consideration (Fish 1999). Victor *et al.* (2004) report that, to a large extent, a herbarium consists of its collections of preserved plant specimens. Angiosperms, seed-bearing plants whose ovules, and hence seeds, develop within the ovary (Leistner 2005), are usually pressed and then stored dried and mounted on sheets of card. In addition to the main collection of pressed, mounted specimens, a herbarium can contain collections of fruits and seeds, bulky specimens, wood samples, bryophytes, fungi, fossils, lichens and plant material stored in preservatives, illustrations, photographs, and copies of specimens, as well as microscope slides. Among the above-mentioned collection types, only the main collection, seed and fruit collection, as well as illustrations, photographs and copies of specimens were utilized for this investigation.

According to Victor *et al.* (2004) the **main collection**, consisting of angiosperms and pteridophytes, may or may not include cultivated plants and gymnosperms. Gymnosperms are seed plants with the ovules borne on the surface of the sporophyll (Leistner 2005). Specimens that have been used for research purposes, for example, anatomical or ecological studies, are deposited in herbaria by the researchers for future reference and are termed voucher specimens.

Seeds, cone and fruit collection is called a **carpological collection** and is housed separately. Each of the seed cone and/or fruit collection is cross-referenced with the corresponding herbarium sheet. Seeds and fruits are kept in boxes or bottles in metal filing cupboards with shallow drawers. It is vital that seeds are kept dry to prevent germination. Fleshy fruit can also be stored in spirits (a liquid chemical). All seeds must have a voucher specimen in the main collection or they are worthless for scientific research (Victor *et al.* 2004).

Similar storage conditions apply to very fleshy or delicate structures or complex flowers, such as those of Asclepiadoideae and Cucurbitaceae. The advantage of this method of storage is that the three-dimensional shape of the specimen is maintained. Plant material preserved in this way can be used for anatomical research and for taking measurements of features that shrink when the plant material is dried. Specimens are usually fixed to prevent their anatomical distortion and then stored in jars with preserving liquid, for example, alcohol or industrial methylated spirits (IMS) (Victor *et al.* 2004).

Victor *et al.* (2004) report that illustrations or photographs are useful in providing a permanent record of the habit, habitat, and colours of the plant. Prints or slides can be used in publications and lectures. They may prove to be of great value if they depict habitats as they were before being destroyed, or species as they were before becoming extinct.

2.1.1.3 Pressing, drying and mounting

The specimens are pressed to flatten the specimen as much as possible, as bulky specimens take up a large amount of space. To prevent wilting, specimens are positioned in such a way that the different parts of the plant are clearly visible as well as to preserve delicate organs without crushing them (Fish 1999, Victor *et al.* 2004). Specifically for Cucurbitaceae, Fish (1999) reports that collected fruits must be sectioned both longitudinally and across as fruit sectioning is one of the diagnostic features important for their identification.

Elimination of specimen moisture content through drying is one of the best preservation methods and enables specimens to last up to hundreds of years. This can be achieved by using three basic drying procedures, namely, natural, field and electrical driers. Natural drying can be achieved by sun-drying the pressed plants (in the press), especially in areas with low humidity. If the

collector is in the field for a long time and/or has collected plants with very high moisture content, field drying is used. Essentially, the press is placed above a heat source, for example, a stove, and warm dry air is allowed to circulate through the ventilators made of corrugated cardboard (Fish 1999).

Electric dryers have different designs: one allows the whole press to be placed on the dryer but for another, the wooden lattice frames are removed while the plant is pressed down with weights, for example, iron weights and the fan-generated warm air is allowed to circulate (Fish 1999).

According to Fish (1999) and Victor *et al.* (2004), mounting allows the best observation of all the characteristics of the specimens and displays all data on labels clearly, in a fixed position. It prevents damage and preserves the specimen by attaching it firmly to strong acid-free paper or boards. The material used for the attachment can be easily removed and replaced to allow better observation of specimens and removal of small portions for more detailed study.

2.1.2 Classification and identification

Plant families and species can be distinguished by one, two or a combination of characteristics that are referred to as the diagnostic family or species characteristics. Some of these characteristics are usually not visible and are difficult to use in practice. However there are some special characteristics that are regularly associated with certain families and these are used for development of dichotomous keys (Siebert 2004). A dichotomous key is defined as a list of characteristics drawn up to enable speedy identification of a plant. Prominent contrasting features are given at each stage in the key so that by a process of elimination, successively smaller groupings of organisms are split off until the specimen can be identified. Simple keys to flowering plants often rely solely on either flower structure or leaf characteristics. The leaf characteristics allow identification during nearly all seasons.

Two types of keys exist, namely, taxonomic keys that use floral parts and simple keys that use field guides. Both keys are used to classify plants belonging to the family Cucurbitaceae, and thereafter *Coccinia palmata* and *Lagenaria sphaerica* species.

2.1.2.1 Family Cucurbitaceae Juss.

Plants belonging to this family are monoecious or dioecious, tendriliferous, annual or perennial herbs, shrubs or undershrubs, prostrate or scandent, rarely erect herbs without tendrils, usually variably hairy, rarely spiny, and sometimes with tuberous or woody rootstock (Andres 2004, Dyer 1975, Jeffrey 1978). Andres (2004) reports that plants are coarse and often scabrous with trichomes containing phytoliths; stems are typically 5-angled, characterised anatomically by bicollateral vascular bundles, often arranged in two concentric rings. Leaves are alternate, extipulate, variable, thus palmately veined, simple or pedately compound, sometimes pinnatifid (e.g. *Citrullus*), sometimes large, rarely undeveloped, with extra-floral nectaries often present (Andres 2004, Dyer 1975, Jeffrey 1978). Andres (2004) and Jeffrey (1978) further report that tendrils are lateral to the petiole base, simple, distally 2-fid (2-/bi-lobed) or proximally 2-7-fid, rarely reduced into spines or absent, usually one at each node, coiling or with adhesive tips.

Flowers are usually unisexual, rarely hermaphrodite, epigynous, monoecious or dioecious, axillary, variously arranged, sometimes paniculate, and often solitary (Andres 2004, Dyer 1975, Jeffrey 1978). Jeffrey (1978) reports that glandular bract-like structures (probracts) are sometimes present at the base of peduncles. Andres (2004) describes the inflorescence as determinate, cymose or solitary, the latter more commonly in the female flower, axillary, small to large, with a shallow to tubular hypanthium; calyx synsepalous with five lobes; and corolla sympetalous with usually five lobes, sometimes petals free, arising from the

hypanthium, actinomorphic or slightly zygomorphic, campanulate, rotate to salverform, often showy but ephemeral, orange-yellow, yellow to white or greenish, less commonly pink, purple, or red.

Male flowers have their calyx or receptacle with a campanulate, funnel-shaped or cylindric tube, (3-4)5(6)-lobed; lobes are free, rarely clawed, sometimes lobed or fimbriate, valvate or the margins involute; stamens (1-2)3(4-5), usually 3, are composed of two double stamens and one single stamen, sometimes with a staminode; filaments are free or joined, usually shorter than the anthers: anthers are free or cohering, 1-2 thecous, sometimes varying in one flower; thecae are straight, curved, or variously bent; and a rudimentary pistil is present or absent (Andres 2004, Dyer 1975). Andres (2004) further reports that key characters of genera are often based upon the morphology of the androecium.

According to Andres (2004), Dyer (1975) and Jeffrey (1978), the female flowers have both a calyx and corolla as in the male flower, with staminode present or absent; the ovary is inferior, more rarely free at the apex, usually 3-locular, sometimes 1-2- or spuriously 4-6-locular, with many, rarely 1-2, ovules in each loculus. Placentation is parietal, rarely axillary, and placentae are often intrusive. Ovules are anatropous, 1-many horizontal, pendulous or ascending; the style is terminal, simple or divided, sometimes surrounded at the base by a disc-like structure; the stigma is various, usually made of 3-fleshy lobes. Fruit is often a berry, fleshy or corky or a dry capsule as well as hard-shelled pepo, usually indehiscent but some are dehiscent and many-seeded. Seeds are often flattened, smooth or pitted, sometimes hairy or winged or variously ornamented or coloured, and often make good key characteristics between closely related species. Some seeds contain edible and/or medicinal seed oil, with the endosperm absent. Andres (2004) states that some indehiscent fruit are adapted to dispersal by floating in rivers and ocean currents, frequently containing bitter purgative cucurbitacins (tetracyclic triterpenoids, bitter-tasting substances) found also in the leaves and roots.

The family is distinct morphologically and biochemically from other families and is therefore considered monophyletic. Generally the opinion is that, phylogenetically, it is most closely allied with the Begoniaceae in the order Violales. A number of genera are not clearly defined and are in need of modern monographic treatments (Andres 2004).

2.1.2.2 Species *Coccinia palmata* (Sond.) Cogn. and *Lagenaria sphaerica* (Sond.) Naud.

Coccinia palmata (Sond.) Cogn. is a perennial climber with stems growing up to 8m (Pooley 1998). Stems are corky with age and tendrils are forked. Leaves are 40-120 mm long, sparsely hairy, with small black dots, are more or less 5-lobed, with toothed, lobed margins. Pooley (1998) reports that male flowers are on a long stem while female flowers are solitary on short stems. Flowers are about 30 mm in diameter, and cream or yellow in colour. The flowering period ranges from December to June. Fruits are red and 50-80 mm x 20-35 mm.

Lagenaria sphaerica (Sond.) Naud. is also perennial. Stems are annual, usually grow up to 10 m long (high) or more, and are minutely puberulous, prostrate or scandent. Leaf-lamina are 5-19 cm x 4-21.5 cm, broadly ovate in outline, cordate, dark green and minutely asperulous above, paler and finely, usually densely puberulous or hispidulous beneath, and palmately 5-lobed. Lobes are shallow to deep, ovate to elliptic, obscurely to usually coarsely sinuate-dentate, often lobulate, obtuse to acute, long-apiculate, and the central lobe is the largest (Jeffrey 1978). He reports that petioles are 1-12 cm long, minutely puberulous; glands are prominent and patent. Probracts are 6-14mm long and rather narrow. Flowers are dioecious and fragrant. Male flowers are racemose and rarely solitary. The peduncle is 1-20 cm long. Bracts are small, 2.5-4 mm long, lobed and dentate. Pedicels are 0.3-5 cm long. The receptacle-tube is 0.9-1.7cm long, obconic below, expanded above, and minutely puberulous; lobes 3-6 mm x 1.8-

2.5mm, remote, broadly lanceolate or triangular-lanceolate, acuminate and glandular. Petals are 2.5-5.5cm x 2-4.5cm, obovate, rounded, and white with green veins. Anthers are oblong or ovate, exserted, and free. Female flowers grow on 1.5-8.5cm long peduncles. The ovary is 12-20mm x 6-15mm, ellipsoid, and densely tomentose. Receptacle lobes and petals are similar to those of the male flowers. Pooley (1998) reports that the flowering period ranges from August to June. According to Jeffrey (1978), the fruit is 7-11cm x 6-10cm, subglobose, smooth, and deep green with small paler spots and also larger scattered paler patches. The fruit stalk is 2.5-10cm long, stout, and expanded at the apex. Seeds are 8.5-11.5mm x 5-6 x 2-2.5mm, oblong, subtruncate and emarginate at the base, slightly narrowed towards the apex, the faces with 2 flat submarginal ridges.

2.1.3 Species ecology and distribution

Cucurbitaceae is primarily found in the warmer regions of the world, including the pantropical and subtropical regions, with a few representatives in temperate to cooler climates (Andres 2004, Chung *et al.* 2003, Jansen van Rensburg *et al.* 2004). This family has plants that grow in grasslands, forest margins as well as in forests. As a result, they grow in the range from full sunlight to full shade conditions (Andres 2004). They are either mesophytic or xerophytic, primarily tropical or subtropical, and have roughly equal distribution in both eastern and western hemispheres. Indigenous cucurbits prefer the most arid areas of western and central southern Africa.

Moss (1994) reports that "the Kalahari and Namib Desert systems represent a major centre of diversity for certain members of Cucurbitaceae, and species and genotypes from these areas could be used in drought-adaptation breeding programmes for cultivated cucurbits". According to Andres (2004) indigenous Cucurbitaceae species are frost-sensitive annuals or perennials, the latter often persisting by means of tuberous storage roots, for example, species of Bryonia

occur in northern Europe, sprouting rapidly from perennial roots. Pollinators are typically bees and moths and sometimes hummingbirds and bats that are attracted to the rich nectar supply and pollen and are guided by the UV-reflective petals. Pollen-gathering insects may be fooled into visiting the female flowers, which have a gynoecium that mimics the structure of the androecium in the male flowers.

Lagenaria sphaerica (Sond.) Naud. is a robust climber reaching the canopy of riverine forest. It occurs in damp, low-lying areas. It is distributed mostly from the eastern Cape to East Africa. *Coccinia palmata* is found in shady places from the eastern Cape to southern Mozambique and in Malawi (Fox and Norwood Young 1982, Pooley 1998).

2.2 Cucurbitaceae species usage

The Cucurbitaceae or cucurbit family, also commonly referred to as the cucumber, gourd, melon, or pumpkin family (Pooley 1998, Andres 2004) is a large to medium-sized plant family (Andres 2004, Jansen van Rensburg *et al.* 2004), with about 119 genera and 850 species (Andres 2004, Berrie 1977, Jeffrey 1978, Pooley 1998). It is amongst the most important plant families supplying humans with edible products and useful fibres (Bisognin 2002). It is also a major family for economically important species, particularly those with edible leaves and/or fruits. Important food plants, some of which are alien to Africa, include squashes, cucumbers, watermelons and luffas (Pooley 1998) while several of these represent some of the earliest cultivated plants in both the Old and New Worlds (Andres 2004). Jansen van Rensburg *et al.* (2004) reports that villagers throughout Africa generally consume leaves, fruits and flowers of cultivated species. Leaves and fruits of some wild cucurbits are also harvested in the veld. Bisognin (2002) further reports that plants of the Cucurbitaceae family are very similar in above-ground development, but they have high genetic diversity for fruit shape and other fruit characteristics, resulting in a variety of uses.

Members of the Cucurbitaceae family are included as one of the Indigenous Leafy Vegetables (ILVs) or Traditional Leafy Vegetables (TLVs), which are used in many countries of sub-Saharan Africa, such as Zimbabwe, Kenya, Senegal, Botswana, Cameroon and South Africa (Ngundam Poubom 1999, Chigumira Ngwerume and Mvere 1999, Chweya and Eyzaguirre 1999, Jansen van Rensburg *et al.* 2004, Matlhare *et al.* 1999, Maundu *et al.* 1999, Seck *et al.* 1999). Traditional vegetables are all categories of plants whose leaves, fruits or roots are acceptable for use as vegetables, while Chigumira Ngwerume and Mvere (1999) define them as those vegetables which have been known and

utilized over generations in Africa although they may not have originated in Africa.

Indigenous vegetables are widely consumed and they can play an important part in alleviating hunger and malnutrition in sub-Saharan Africa, but they are often neglected in research. They are important sources of micronutrients, including vitamins A and C, iron and other nutrients and are sometimes better nutritional sources than the "modern" vegetables. ILVs are crucial to food security, particularly during famine and natural disasters, two scourges that are currently prevalent in sub-Saharan Africa. Many of these plants are growing in the wild or as weeds in cultivated areas, but have also been domesticated through semi-cultivation or cultivation. Being accessible to the low-income communities in rural and urban areas, they offer an opportunity of improving the nutritional status of many poor families whose health and nutrition are at risk.

According to Maundu *et al.* (1999), most of the ILVs grown up to now in Kenya are regarded as valuable especially because they have significant cultural value attached to them. Cultural beliefs and practices play a major role in the conservation of leafy vegetables. Leafy vegetables are believed to add vigour and vitality to the diets of those who eat them frequently and are recommended to pregnant and lactating mothers so as to improve the blood supply of their infants. Particular beliefs and practices support the growing and management of leafy vegetables, such as the beliefs of adherents to the Seventh Day Adventist Church of encouraging a vegetarian diet. Further, ILVs are recommended as the best substitutes of meat, as they are more tasty and filling than the exotic vegetables. Economically, ILVs are thus the most cost-effective relishes for most diets.

Some cucurbits have medicinal values, for example, in treating diabetes, high blood pressure, skin complaints, influenza, as a diuretic, and other uses. Pooley (1998) notes that medicinal cucurbitaceae include African Cucumber (*M.*

balsamina), Wild Cucumber (*C. palmata*), and Wild Melon (*L. sphaerica*), to name a few. Though this plant family is regarded as having useful species, some species are reported to be very toxic, especially the fruits (Hutchings 1996, Watt and Breyer-Brandwijk 1962).

Coccinia palmata is commonly used in traditional medicine (FAO 1988, Pooley 1998). Dried infusions are administered as enemas or taken as purgatives (Hutchings 1996). Pooley (1998), Jacobs (2002), Fox and Norwood Young (1982) and FAO (1988) report that Zulus eat the cooked leaves of this vine as a vegetable. The roots of this species, which have a flavour resembling potato, are eaten by the local people in Malawi in times of famine. Watt and Breyer-Brandwijk (1932, 1962) state that the Nyanjas of Nyasaland give an infusion of the root of this species to infants for gastric upset.

Watt and Breyer-Brandwijk (1962) record that the Zulu use an infusion of the root and of the leaves of *Lagenaria sphaerica* for stomachache. Hutchings (1996) and Pooley (1998) state that the infusion made from the mixture of *L. sphaerica* leaves and *Bidens pilosa* roots is used for stomachaches. Pounded root decoctions of *L. sphaerica* are used for treating swelling caused by blood disorders. The fruit is reported to be an ingredient in a Xhosa remedy for glandular swellings (Hutchings 1996). According to Peters *et al.* (1992) leaves are edible while Williamson (1975) reports that they are eaten as *ndiwo* (traditional Venda dish consisting of a mixture of edible leaves and mielie meal). Williamson (1975) further reports that the fruits of *L. sphaerica* are cut open and used as a soap substitute. The fruits are hung in pigeon cotes to encourage the birds to lay. Mature fruits are sold in South Africa for medicinal use (Andres 2004).

2.3 Polyploidy (Chromosome doubling)

Polyploidy is defined as the possession of three or more complete sets of chromosomes in a cell (Ramsey and Schemske 1998), and an increased number of genomes in a cell (Broertjies and van Harten 1988). According to Ramsey and Schemske (1998), polyploidy is an important feature of chromosome evolution in many eukaryote taxa. Yeast, insects, amphibians, reptiles and fishes are known to contain polyploid forms and recent evidence of extensive gene duplication suggests that the mammalian genomes have a polyploid origin.

In plants, polyploidy represents a major mechanism of adaptation and speciation. "Polyploidy is important in the evolutionary history of plants, and has played a crucial role in shaping the genome structures of all eukaryotes" (Mable 2003). According to Snyder *et al.* (1985), polyploidy is widespread in certain plant groups and it occurs in many valuable crop plants, such as wheat, oats, cotton, potato, banana, coffee and sugar cane. Among flowering plants, at least one-third of the existing species originated as some form of polyploid. Martelotto *et al.* (2005) report that angiosperms (flowering plants) in particular have been the subject of considerable speculation about the frequency of polyploidy occurrence.

Lawton-Rauh (2003), Ramsey and Schemske (1998) and Martelotto *et al.* (2005) mention that it is estimated that between 47% to 70% of angiosperm species are polyploid. However, Sliwinska and Lukaszewska (2005) report that over 90% of angiosperms have undergone cell polyploidisation through either endomitosis or endoreduplication. Up to 95% of pteridophytes are polyploids (Lawton-Rauh 2003). Estimates vary regarding the proportion of pteridophytes that have experienced one or more episodes of chromosome doubling at some point in their evolutionary history. Differences in ploidy have been observed among related congeners and even within populations of taxonomic species, and there

is evidence that individual polyploid taxa may have multiple origins. These observations suggest that polyploid evolution is an ongoing process and not a rare macro-evolutionary event.

Research in agricultural and natural systems indicates that polyploids often possess novel physiological and life-history characteristics not present in the progenitor cytotype. Some of these new attributes may be adaptive, allowing a plant to enter a new ecological niche. Because plants of different ploidies are often reproductively isolated by strong post-zygotic barriers, polyploidy is also one of the major mechanisms by which plants evolve reproductive isolation.

Bisognin (2002) and Chung *et al.* (2003) report that *Lagenaria* and *Coccinia* genera have the basic haploid chromosome number (haploid component) of 11 (2n=22) and 12 (2n=24), respectively. Chung *et al.* (2003) further reports that the haploid numbers 11 and 12 are common to the rest of Cucurbitaceae genera.

2.3.1 Chimeras

Plants in which the cell population contains cells varying in chromosome number is described as mixoploid, a term which embraces all types of mosaics and chimeras so long as there is heterogeneity in the chromosome constitution (Tilney-Bassett 1986, Rieger *et al.* 1976, Koutoulis *et al.* 2005). Mixoploids, which result largely from polyploidy in which there is a clear development into a sectorial or mericlinal chimera and eventually into a stable periclinal chimera, are called cytochimeras (Tilney-Bassett 1986).

Rieger *et al.* (1976) report that sectorial chimeras have different tissues growing side by side and occupy distinct sectors of varying size. A mericlinal chimera occurs when the inner tissue of a particular genetic constitution is only partly surrounded by the outer tissue of a different genetic constitution. Periclinal

chimera has the inner tissue layer completely surrounded by the outer layer with a different genetic constitution.

According to Rieger *et al.* (1976), in plants where the apical meristem is composed of three tissue layers (L I, L II and L III), four different types of periclinal chimeras are distinguishable: (1) Haplochlamydeous periclinal chimeras in which only the outermost (L I) of the three layers is idiotypically different from the others, (2) Diplochlamydeous periclinal chimeras in which only the innermost (L III) of the three layers is idiotypically different from the others, (3) Mesochimeras in which only the middle layer (L II) is idiotypically different from the other two layers and (4) Trichimeras in which all three layers are idiotypically different from each other.

Koutoulis *et al.* (2005) report that the use of the genome doubling agent, colchicine, in inducing tetraploids from diploid material may result in mixoploids (chimeras consisting of diploid and tetraploid tissue) since plant meristem consists of many cells and may not be similarly affected by colchicine. Flow cytometry was used to determine the DNA ploidy level of different tissues of individual hop plants following colchicine treatment of diploid hops. Plants that are shown to be mixoploid after analysis of leaf tissue can display higher levels of tetraploid nuclei in root tissue, such that they can be mistakenly classified as tetraploid.

2.3.2 Mechanism of polyploid formation

Several cytological mechanisms are known to induce polyploidy in plants. Somatic doubling in meristem tissue of juvenile and adult sporophytes has been observed to produce mixoploid chimeras. Somatic polyploidy is known to be common in many non-meristematic plant tissues. For example, normal diploid *Vicia faba* contains tetraploid and octoploid cells in the cortex and pith of the stem. Such polyploid cells occasionally initiate new growth, especially in wounds

or tumors, and are a potentially important source of new polyploid shoots. The frequency of endopolyploidy, and the relative likelihood of polyploid formation from different endopolyploid tissues are not well known (Tilney-Bassett 1996, Ramsey and Schemske 1998, Yang and Loh 2004, Rieger *et al.* 1976).

Ramsey and Schemske (1998) report that "somatic doubling can also occur in a zygote or young embryo, generating completely polyploid sporophytes. This phenomenon is best described from heat shock experiments in which young embryos are briefly exposed to high temperatures. Corn plants exposed to 40°C temperatures approximately 24 h after pollination produced 1.8% tetraploid and 0.8% octoploid seedlings. Polyploid seedlings are also known to arise from polyembryonic ("twin") seeds at a high frequency but it is now believed that such polyploids are generally of meiotic rather than somatic origin. In general, little is known about the natural frequency neither of somatic doubling in plants nor of the effects of interspecific hybridization on its occurrence".

According to Ramsey and Schemske (1998) and Rieger *et al.* (1976) a second major route of polyploid formation involves gametic nonreduction, or meiotic nuclear restitution, during micro- and megasporogenesis. This process generates unreduced gametes, also referred to as 2n gametes, which contain the full somatic chromosome number. The union of reduced and unreduced gametes, or of two 2n gametes, can generate polyploid embryos. Diploid (2n) gametes have been identified in many plant taxa. Polyspermy, the fertilization of an egg with more than one sperm nucleus, is known in many plant species, and has been observed to induce polyploidy in some orchids. However, it is generally regarded as an uncommom mechanism of polyploid formation.

Unreduced gametes are believed to be a major mechanism of polyploid formation (Lawton-Rauh 2003, Broertjies and van Harten 1988, Ramsey and Schemske 1998). Both 2n pollen and 2n eggs have been observed in hybrid and non-hybrid agricultural cultivars and natural plant species. Unreduced pollen

grains can often be identified by size, as they typically have a diameter 30-40% larger than that of reduced pollen, and the distribution of pollen size in plants known to produce 2n pollen is often bimodal. Unreduced female gametophytes can sometimes be identified by size, but more often the frequency of 2n gametes is indirectly estimated using controlled, interploidy crosses in plants with very strong interploidy crossing barriers. The progeny generated are usually the products of 2n gametes.

Ramsey and Schemske (1998), distinguish between somatic doubling and 2n gametes as mechanisms of polyploid formation. This requires a system of genetic markers and a detailed knowledge of the cytological mechanism of gametic non-reduction, which are seldom available. There is, however, strong circumstantial evidence that 2n gametes are often involved in polyploid formation. The parents of spontaneous polyploids have, upon cytological analysis, commonly been found to produce 2n gametes. Conversely, plants known to produce 2n gametes can be crossed to produce new polyploids.

In many cases, spontaneous polyploids have cytotypes that appear to have been formed by the union of reduced and unreduced gametes rather than by somatic mutation. Spontaneous polyploidy formation generally doubles the base chromosome number. For example, triploids and pentaploids are found in the progeny of open-pollinated diploid *Crepis capillaris*, and these appear to have been produced by the union of reduced (n) and unreduced (2n and 4n) gametes. Similarly, triploids generated by backcrossing diploid hybrid *Digitalis ambigua* x *purpurea* are thought to have arisen from unreduced gametes produced by this interspecific hybrid.

2.3.3. Factors affecting or enhancing polyploidy

The environmental and chemical factors that enhance the formation of polyploidy are discussed below:

a) Environmental factors enhancing polyploid formation

According to Ramsey and Schemske (1998) and Broertjies and van Harten (1988), several researchers have found that 2n pollen is stimulated by environmental factors such as temperature, herbivory, wounding, and water and nutrient stress. Temperature, and specifically, variation in temperature, has particularly large effects. A dramatic increase in 2n pollen production in field and greenhouse cultures of *Strizolobium* sp., *Datura stramonium*, and *Uvularia grandiflora* was observed by Ramsey and Schemske (1998) to follow aberrant cold spells. Potato genotype selected for the tendency to undergo gametic non-reduction had approximately twice the mean frequency of 2n pollen in coastal fields as compared to in a greenhouse. This effect was attributed to the temperature differences of the two environments. The frequency of 2n pollen in randomly selected *Achillea millefolium* plants reared in a temperature-cycling growth chamber was approximately six times that in the natural population from which the study plants had been sampled.

Plant nutrition, herbivory, and disease may also affect 2n gamete production. The rate of polyploid production per flower in F_1 *Gilia* hybrids grown in low-nutrient conditions was almost 900 times greater than that of plants grown in high-nutrient conditions, a result attributed to poor pairing at meiosis in the low-nutrient treatment. However the high level of polyploid production per flower in the low-nutrient treatment was partially offset by a much lower flower number, such as the number of polyploids produced per plant which was only seven-fold greater (Ramsey and Schemske 1998).

Ramsey and Schemske (1998) report that many of the environmental factors known to influence 2n gamete production are experienced by plants in their natural habitats. This suggests that natural environmental variation, as well as large-scale climate change, could substantially alter the dynamics of polyploid evolution. The high incidence of polyploidy at high latitudes, high altitudes, and recently glaciated areas may be related to the tendency of harsh environmental conditions to induce 2n gametes and polyploid formation.

b) Chemical factors enhancing polyploid formation

Broertjies and van Harten (1988) report that chemicals like ethyl methanesulphonate (EMS) are known to mutate active genes with a higher frequency than inactive genes in *Escherichia coli* (*E. coli*). In addition, repetitive or heterochromatic DNA regions are thought to be hot spots for the effects of certain chemicals. The use of chemical mutagens, like EMS and many other compounds, induce more point mutations and few chromosome aberrations. For seed propagated crops, chemicals are often the most efficient mutation inducing agents. Often, chemicals may induce a somewhat different mutation spectrum than irradiation. The use of chemical mutagens, like EMS and methyl methanesulphonate (MMS), *in vitro* on callus cultures of sugarcane has been reported.

A survey by the International Agricultural Exchange Association (IAEA) on the number of plant cultivars derived from mutation induction showed that only in five cases had successful use been made of chemical mutagens, colchicine-induced polyploids excluded. Those cases refer to apple, carnation and rose. These meagre results sufficiently illustrate the relative lack of importance of chemical mutagens from a practical point of view for vegetatively propagated plants up to now. The fast-increasing application of different *in vitro* techniques may change

this situation drastically in favour of chemical mutagens (Broertjies and van Harten 1988).

Broertjies and van Harten (1988) also report that chemical mutagens are difficult to apply when vegetative parts are involved. They have described a technique of injecting perennial trees by means of a syringe. In most cases, however, existing buds on scions, stem cuttings, tubers, bulbs and rhizomes are treated, either by immersion of the plant part in the mutagenic solution or applying the mutagens via a cotton plug placed on the buds. The same procedure is applied when mutations are to be obtained in the area from which adventitious buds can be induced.

Colchicine is a water soluble alkaloid extracted from seeds and corms of *Colchicum automnale* L. species (Broertjies and van Harten 1988, Hutchings 1991, Tilney-Bassett 1986). Colchicine is universally used to artificially induce plant polyploidy. In nature, polyploids usually arise through the union of unreduced gametes, whereas colchicine inactivates the spindle of a dividing cell and arrests mitosis at metaphase. The chromosome divides, but with no spindle, daughter chromatids cannot be drawn to opposite poles, so they remain at the equator of the cell, where they become enclosed in a common nuclear membrane instead of separating into two daughter cells (Tilney-Bassett 1986, Hutchings 1991, Broertjies and van Harten 1988, Snyder *et al.* 1985). As it only affects actively dividing cells, colchicine is generally applied to the actively growing meristematic regions of young plants or to germinating seedlings (Tilney-Bassett 1986).

Tilney-Bassett (1986), Rieger *et al.* (1976) and Snyder *et al.* (1985) further report that if meristematic cells have prolonged contact with colchicine, the chromosome may pass through a further cycle of DNA synthesis and replication before once again becoming arrested at the next metaphase. In the first round of chromosome division diploid cells therefore become tetraploid (four sets of

chromosomes), and in the second round the tetraploid cells, in turn, become octoploid (eight sets of chromosomes). The method of inducing polyploidy using colchicine is referred to as colchiploidy (Tilney-Bassett 1986, Rieger *et al.* 1976).

According to Tilney-Bassett (1986), Snyder *et al.* (1985) and Beck *et al.* (2003), the effectiveness of colchicine depends on its concentration and exposure time to the treated tissues. Colchicine has been applied to plant tissues as a solution in water at a concentration varying between 0.01 and 1.00% (m/v). As colchicine is rather toxic, overdosing kills tissues, and the ideal dose for the plant in question has to be found by trial and error. Beck *et al.* (2003) report that mortality increases with an increase in colchicine concentration as well as with an increase in exposure time.

2.4 Seed viability

According to Bradbeer (1988), a viable seed is one which is capable of germination under suitable conditions. Bradbeer (1988), Bewley and Black (1985), Raven *et al.* (1992) and Raven *et al.* (1999) report that some seeds may remain viable for a long time in a dormant condition, enabling them to exist for many years, decades, and even centuries under favourable conditions. A 1288 year-old sacred lotus (*Nelumbo nucifera*) and at least 10 000 year-old arctic lupine (*Lupinus arcticus*) seeds are known to have germinated within 48 hours. Various enzyme systems may progressively fail in the stored seeds, eventually leading to a complete loss of viability.

According to Bradbeer (1988) and Bewley and Black (1985) the majority of seed species retain their viability when dried. Drying is, in fact, the normal final phase of maturation of most seeds. Hence, it is common for seeds to be stored in a "dry" state or more correctly, with a low moisture content. There are, however, some seed species, called recalcitrant seeds, that must retain a relatively high moisture content during storage in order to maintain maximum viability.

2.4.1 Effect of temperature and moisture during storage on seed viability

Bradbeer (1988) and Bewley and Black (1985) report that seeds which can be stored in a state of low moisture content are called orthodox seeds. Their viability under certain conditions conform to some general rules as follows: (a) for each 1% decrease in seed moisture content the storage life of the seed doubles; (b) for each 5.6°C (10°F) decrease in seed storage temperature the storage life of the seed is doubled; and (c) the arithmetic sum of storage temperature in degrees F and the percent relative humidity (RH) should not exceed 100, with more than half the sum contributed by the temperature. These rules clearly indicate that temperature and moisture content of the seed are major factors in determining viability in storage.

Victor *et al.* (2004) report that seeds may be stored for future cultivation or re-introduction for conservation purposes, in which case special conditions are needed to maintain their viability. For short-term storage (days, weeks, or months), seeds may be kept at 5°C in a normal refrigerator inside an airtight container with silica crystals to absorb moisture. Long-term storage (longer than one year) requires seeds to be stored at –20°C to –5°C. Stored seeds should not be exposed to warmth and moisture, or they will germinate or become non-viable.

a) Moisture content

According to Bradbeer (1988) and Bewley and Black (1985) moisture content is defined by International Seed Testing Association (ISTA) practice as:

$$\% \text{ Moisture content} = \frac{\text{Fresh weight of seed} - \text{dry weight of seed}}{\text{Fresh weight of seed}} \times 100$$

In general, when the moisture content is high (>30%), non-dormant seeds may germinate. With moisture content 18-30%, rapid deterioration by microorganisms can occur. Seeds at moisture content greater than 18-20% will respire, and in poor ventilation the generated heat will kill them. Below 8-9% moisture content, there is little or no insect activity, and below 4-5% moisture content seeds are immune from attack by insects and storage fungi, but they may deteriorate faster than those maintained at slightly higher moisture content.

The activities of seed storage fungi are ultimately more influenced by the relative humidity (RH) of the interseed atmosphere than by the moisture content of the seeds themselves. This is because the moisture content of some seeds (such as oil seeds) may be different from that of others (such as starchy seeds) even though both are in equilibrium with the same atmospheric RH. For example, all

cereals and many of the legumes that are high in starch and low in oil content have a moisture content of about 11% at 45% RH, whereas oil-containing seeds (such as rape) have a moisture content of only 4-6% at this RH.

b) Temperature

Cold storage of seeds at 0-5°C is generally desirable. The seeds, however, should be sealed in moisture-proof containers or stored in a dehumified atmosphere, otherwise the RH of storage could be high, causing the seeds to gain moisture. If they are brought out to a higher temperature (such as for transport), they might deteriorate because of their high moisture content. At moisture content below 14% no ice crystals form within the cells upon freezing, so storage of dry seeds at subzero temperatures after freezing in dry atmosphere should improve longevity. Freeze-drying of certain seeds improves their longevity in storage, but others may be killed by this treatment. High temperature during drying, or drying too quickly or excessively, can dramatically reduce viability. A method that is increasing in popularity, particularly for small batches of seeds for gene banks, is to keep them immersed in liquid nitrogen. Under this condition seeds should survive indefinitely (Bradbeer 1988, Bewley and Black 1985).

2.4.2: Effect of pre- and post-harvest conditions on seed viability during storage

Environmental variation during seed development usually has little effect on the viability of seeds, unless the ripening process is interrupted by premature harvesting. Variation in the environment of seeds at about the time of completion of maturation and harvesting can result in different potential viability periods. Viability of cereal grains is generally lower in years when ripening and harvesting conditions are poor (Bradbeer 1988, Bewley and Black 1985).

Nerson (2002) reports that germinability (percentage and rate of germination) of immature, half-mature and mature watermelon seeds was examined over 10 years of storage at 10°C and 45% RH. Immature seeds had very poor germinability. Germinability of all seed lots increased during the first 0.5-5 years of storage. Mature seeds retained their high germinability till the end of the 10 years storage period, whereas the germinability of half-mature and immature seeds declined after 5-6 years of storage.

Mechanical damage inflicted during harvesting can severely reduce the viability of some seeds, such as certain large-seeded legumes. Cereals are largely immune from mechanical injury, presumably because of the protective outer structures, the palea and lemma. Small seeds tend to escape injury during harvest, and seeds that are spherical tend to suffer less damage than elongated or irregularly shaped ones. During storage, injured or deeply bruised areas may serve as centers for infection and result in accelerated deterioration. Injury close to vital parts of the embryonic axis or near the point of attachment of cotyledons to the axis usually bring about the most rapid losses of viability (Bradbeer 1988, Bewley and Black 1985).

2.5. Seed germination

Seed germination undergoes three phases. The first phase or imbibition phase is largely a consequence of matric forces and water uptake regardless of whether the seed is dormant or non-dormant, viable or non-viable (Bradbeer 1988, Bewley and Black 1985). This water absorption greatly increases the volume of the seed (sometimes as much as 200 percent) (Keeton *et al.* 1993).

Bradbeer (1988) and Bewley and Black (1985) further report that a wetting front is formed as water permeates the seed and there is an abrupt boundary of water content between wetted cells and those about to be wetted. Moreover, the average water content of the wetted area increases as a function of time. This initial pattern of water uptake is thus marked by three characteristics, namely, a sharp front separating wet and dry portions of the seed; continued swelling as water reaches new regions and an increase in water content of the wetted area. Imbibition is probably very rapid into the peripheral cells of the seed and into a tissue as small as the radicle. Hence, metabolism can commence during this first phase within minutes of introduction of the seed to water.

Phase two is a lag phase in water uptake. During this phase major metabolic events take place in preparation for radicle emergence from non-dormant seeds. Dormant seeds are also metabolically active at this time.

Phase three is concurrent with radicle elongation. Although dormant seeds may achieve phase two, only germinating seeds enter this third phase. The increase in water uptake is initially related to the changes that cells of the radicle undergo as they extend, marking the completion of germination. Then water uptake is influenced by decreases in osmotic potential due to production of low-molecular-weight, osmotically active substances resulting from post-germinative hydrolysis of food reserves. Endosperms and nonpersistent (hypogeal) cotyledons do not

expand and hence do not achieve phase three of water uptake; eventually their water content declines as degeneration occurs (Bradbeer 1988, Bewley and Black 1985).

According to Bradbeer (1988), the duration of each of these phases depends on certain inherent properties of the seed for example, hydratable substrate levels, seed coat permeability, seed size, oxygen uptake, as well as on the prevailing conditions during hydration such as temperature, moisture levels and composition of substrate. Different parts of a seed may pass through these phases at different rates, such as an embryo or axis located near the surface of a large seed may commence elongation (i.e. enter phase three of water uptake) even before its bulky storage tissue has become fully imbibed (i.e. completed phase one). As specific example, when the water content of the whole dent corn (*Zea mays*) grain reaches 75%, the water content of the embryo on a dry weight basis is 261%, but that of the remainder of the grain is only 50%.

According to Keeton *et al.* (1993), the hypocotyl (with attached radicle) is the first part of the embryo to emerge from the seed. The radicle turns downward no matter what the orientation of the seed may be. By the time the epicotyl begins its rapid development, the radicle has already formed a young root system capable of anchoring the plant to the substrate and absorbing water and minerals.

Keeton *et al.* (1993) and Raven *et al.* (1999) report that in some dicots, such as Cucurbitaceae, the upper portion of the hypocotyl elongates and forms an arch, which pushes upward through the soil and emerges into the air. Once the hypocotyl arch is exposed to light, it straightens and thus pulls the cotyledons and the epicotyl out of the soil. The epicotyl then begins to elongate. In some plants, such as squash (*Cucurbita maxima*), the cotyledons become green as they are exposed to light and therefore become important photosynthetic organs. In these dicots, the shoot of the mature plant is mostly of epicotyl origin but a

short region (usually little more than one centimeter) at the base of the stem is derived from the hypocotyl. This type of seed germination, in which the hypocotyl is carried above the ground level, is called epigeous.

Raven *et al.* (1999) further report that during germination and subsequent development of the seedling, the food stored in the cotyledons is digested and the products are transported to the growing parts of the young plant. The cotyledons gradually decrease in size, wither, and eventually drop off. By this time, the seedling has been established and no longer depends upon the stored food of the seed for its nourishment.

2.5.1 Factors affecting seed germination

Embryo growth is usually delayed while the seed matures and is dispersed. Resumption of growth of the embryo, or germination of the seed, is dependent upon many factors, both external and internal. Important external or environmental factors are water, oxygen, light and temperature (Navarro and Guitián 2003, Raven *et al.* 1992 and 1999).

2.5.1.1 External factors: water, oxygen, temperature and light

Most seeds are normally extremely dry with only 5-20% moisture content. Germination is not possible unless the seed imbibes enough water required for metabolic activities. Characteristic patterns of cell enlargement and cell division during embryo development is followed by further growth which requires a continuous supply of water and nutrients (Bradbeer 1988, Raven *et al.* 1992 and 1999).

Excess water lowers the germinability in some seeds. Nerson (2002) reports that poor germinability of immature watermelon (Cucurbitaceae) seeds may be due to the penetration of excess water into both the young embryo and the seed cavity,

which would impede the exchange of gases, like oxygen, involved in the biochemical pathways of the germination process.

According to Bradbeer (1988) and Raven *et al.* (1992 and 1999), glucose breakdown during the early stages of germination may be entirely anaerobic, but as soon as the seed coat is ruptured, the seed switches to respiration, which requires oxygen. If the soil is waterlogged, oxygen availability may be inadequate for aerobic respiration and the seed will fail to grow into a seedling. Nerson (2002) reports that an increase in the oxygen content causes an increase in the germination of muskmelon (Cucurbitaceae) seeds under unfavourably low temperatures.

Seeds from many plant species normally germinate within a certain temperature range (such as 5°C – 45°C) and have a specific optimum temperature for example between 20°C – 30°C (Bradbeer 1988, Navarro and Guitián 2003, Raven *et al.* 1992 and 1999).

Many seeds germinate in the dark. Some seeds, such as lettuce (*Lactuca sativa*) and many weeds, commonly require exposure to light for germination. This requirement is true of many small seeds, which must germinate in loose soil and near the surface in order for the seedling to emerge (Bradbeer 1988, Navarro and Guitián 2003, Raven *et al.* 1992 and 1999).

2.5.1.2 Internal factor: dormancy

The internal factors affecting seed germination include seed dormancy (Navarro and Guitián 2003, Raven *et al.* 1992 and 1999). Even when external factors are favourable, some seeds will fail to germinate and they are said to be dormant (Bradbeer 1988, Raven *et al.* 1992 and 1999). According to Bradbeer (1988), some seeds are born dormant, some acquire dormancy and some have dormancy thrust upon them. These forms of dormancy are called innate, induced

and enforced dormancy, respectively. Innate dormancy occurs when the seeds are in a dormant state on release from the parent plant. The main causes of innate dormancy in seeds are physiological immaturity of the embryo and impermeability of the seed coat to water and gases. Physiologically immature seeds must undergo a complex series of enzymatic and biochemical changes, collectively called after-ripening, before they will germinate (Bradbeer 1988, Navarro and Guitián 2003, Raven *et al.* 1992 and 1999).

The induced dormancy is used to describe the situation in which dormancy develops in response to external and internal cues after release from the parent plant. Enforced (imposed) dormancy is attained when one or more of the basic germination requirements (such as moisture, suitable temperature and aerobic atmosphere) are not met and germination will fail to occur (Bradbeer 1988, Nerson 2002, Raven *et al.* 1992 and 1999).

According to Bradbeer (1988) and Nerson (2002), dormancy is of great survival value to the plant by ensuring through after-ripening that the conditions will be favourable for growth of the seedlings. Some seeds must pass through the digestive tract of birds or mammals before they will germinate. Some desert species will germinate only when inhibitors in their coats are leached away by rainfall. In some seeds the coat must be abraded (Bradbeer 1988) or removed (Nerson 2002) to improve germination e.g. muskmelon (Cucurbitaceae). Temperature increase (fires) and light changes (removal of forest canopy) can also stimulate germination.

Improved germination of watermelon (Cucurbitaceae) seeds after several years of storage at 10°C and 45% RH as compared with that of fresh seeds indicated that these seeds had some degree of dormancy. Seed dormancy in wild species enhances survival. In *Citrullus colocynthis* (Cucurbitaceae), a species closely related to watermelon, fresh seeds were reported to be fully dormant and they reach their maximum germination only after 8 years of storage (Nerson 2002).

2.5.1.3 Effect of polyploidy on seed germination

The treatment of seeds with polyploidy-inducing agents such as colchicine also has a great impact on the seed germination percentage, morphology and physiology of the produced seedlings (Takamura and Mayajima 1996, Beck *et al.* 2003). Colchicine treatment of *Cyclamen persicum* Mill. (Takamura and Mayajima 1996) and *Acacia maemsii* de Wild (Beck *et al.* 2003) seeds, was detrimental to their germination because of increased mortality. This effect worsened with increased concentrations and longer treatments (exposure time) and no polyploid was induced. Tilney-Bassett (1986) reports that colchicine is toxic and overdosing kills the tissues.

2.6. Seedling development and plant growth

Germination of seeds is usually followed by an increase in length and fresh weight of the radicle (Bewley and Black 1985). There are some seeds, however, where the hypocotyl is the first structure to emerge e.g in some members of Palmae such as the date palm and Chenopodiaceae such as spinach. Seedlings can be divided into two types: (1) epigeal, in which the cotyledons are raised out of the soil by expansion of the hypocotyl and often become foliate and photosynthetic (Cucurbitaceae species have this type of seedling); (2) and hypogeal, in which the hypocotyl remains short and compact, the cotyledons remain beneath the soil and the epicotyl expands to raise the first true leaves out of the soil.

An interesting pattern of growth is shown by certain species of *Peperomia* (a dicot) in which one cotyledon emerges from the seed and the other remains as an absorptive organ. It has been suggested that the monocotyledonous condition evolved from this pattern of emergence (Bewley and Black 1985).

The development of the first true leaf marks the end of seedling growth and the formation of the true plant. The plant grows until it flowers and bears fruits. After reproduction, some plants die (annuals) or enter another growth period and reproductive phase (perennials) in a continuous cycle.

2.6.1 Effect of light intensity on aspects of physiology, photomorphogenesis and yield

All life on earth is supported by the radiant energy from the sun, which plants convert into chemical energy by the process of photosynthesis. Light is one of the most important and variable components of the plant environment. The autotrophic plants are directly influenced by the intensity of light, which drives photosynthesis, and thereby provides the chemical energy and carbon needed

for plant growth and development. Thus, an alteration in light intensity, whether a deficit or excess, will result in a disruption of plant metabolic processes. Besides affecting photosynthetic rate, solar radiation also affects plant temperature and photomorphogenic responses in ways that can produce stress (Hale and Orcutt 1987).

An important feature of phytochrome in plants growing in a natural environment is the detection of shading by other plants. Radiation of wavelengths below 700 nanometers is almost completely reflected or absorbed by vegetation, whereas that between 700 and 800 nanometers (in the far-red range) is largely transmitted. Thus, plants grown in shade receive more of far-red light than red light. This causes a dramatic upward shift in the equilibrium ratio of P_r to P_{fr} (that is, more P_{fr} is converted to P_r) in shaded plants and results in a rapid increase in the rate of internodal elongation. Given the competition for light under the forest canopy, a plant's ability to sense the level of light and adjust its growth accordingly has obvious adaptive significance (Morelli and Ruberti 2002, Raven *et al.* 1992 and 1999).

Morelli and Ruberti (2002) report that several studies have suggested that auxins, brassinosteroids (BRs) and gibberellins (Gas) are all involved in the regulation of photomorphogenic processes. These hormones also influence plant stature and organ size, regulating cell division and/or cell expansion. Auxin is involved in diverse developmental processes including cell enlargment, vascular tissue differentiation, root initiation, gravitropic and phototropic responses, and apical dominance.

According to Gardner *et al.* (1985), light has a pronounced effect on stem growth. The internodes of shaded plants, such as in dense stands, are more etiolated (elongation of internodes). The shade effect is believed to be due to auxin enhancement, probably acting synergistically with GA. Theoretically,

photodestruction of auxin is less in shaded stands, since high irradiance decreases auxin and plant height.

Morelli and Ruberti (2002) further report that indole-3-acetic acid (IAA) is synthesized in young leaves of the shoot and transported downwards to the root tip through the vasculature. IAA is also synthesized in other plant organs such as cotyledons, expanding leaves and roots. It has been suggested that auxin might act as a coordinator of growth because it is the only hormone transported polarly along the plant. It has been shown that polar auxin transport (PAT) is affected in a light-dependent manner, and an increasing body of evidence suggests intimate interactions between phytochrome and auxin signaling.

It has been found that auxin and the PAT system are components of the elongation process induced by shade. Consistent with the role of auxin transport direction changes in elongation growth responses to directional light sources and alterations in the perceived gravity vector. In the case of tropic responses, lateral transport of auxin across gravity or light-stimulated plant tissues drives differential growth (Morelli and Ruberti 2002).

The dark-to-light transition of the seedling results in the activation of PAT system in different cell types. The activation is likely to produce a transient reduction of the auxin pool which, in turn, triggers the initial expansion of cotyledons and inhibits hypocotyl elongation. It has been proposed that exposure of autotrophic seedlings to shade light produces a reduction of PAT through the central cylinder. This reduction is also consistent with the inhibition of secondary vascular growth observed in the hypocotyls of seedlings (Morelli and Ruberti 2002).

A decrease in PAT in the central cylinder should also produce a decrease in the auxin concentration reaching the root, resulting in a reduction of lateral root formation and slower growth of the main root. Primary root growth, lateral root

formation and secondary vascular growth are all inhibited. It can be concluded that auxin has been implicated as a coordinating signal in the overall plant response to shade (Morelli and Ruberti 2002).

Dark-grown seedlings are thin and pale. They have longer internodes and smaller leaves than light-grown seedlings. The group of physical characteristics exhibited by these dark-grown seedlings, known as etiolation, has survival value for the seedlings because it increases their chances of reaching light before their stored energy supplies are exhausted. When the seedling tips emerge into the light, the etiolated growth pattern gives away to normal plant growth. The light-triggered growth and developmental responses are called photomorphogenic responses (Gardner 1985, Bradbeer 1988, Raven *et al.* 1992, Raven *et al.* 1999).

The effect of pre- and post-anthesis shading (20% of incident radiation) on pericarp development, cotyledon cell number and seed growth dynamic of fruit from three positions in the capitulum (peripheral, mid and central) of two sunflower (*Helianthus annus* L.) genotypes were studied by Lindstrom *et al.* 2005. Both shading treatments reduced pericarp weight, fruit volume and total yield per plant. Plants shaded during pre-anthesis maintained the number of filled fruits but reduced their individual weight and cotyledon cell number in the three positions on the capitulum. In contrast, post-anthesis shading reduced the number of filled fruits but their individual weight and cotyledon cell number were reduced only in the central fruits (Lindstrom *et al.* 2005).

2.6.2 Effect of polyploidy on plant morphology, physiology, yield and fertility

Polyploidy has a great impact on the whole plant system through its effects on morphology, physiology, yield, fertility and phenology.

An increase in the number of genomic complements is often associated with the emergence of novel phenotypes not present in diploid progenitors (Martelotto *et al.* 2005). Polyploidy usually results in an increase in cell volume and therefore plant parts (Tilney-Bassett 1986, Hannweg and Vos 2001, Sliwinska and Lukaszewska 2005, Snyder *et al.* 1985). Polyploids are expected to have at least one of the following characteristics: larger tuber, rhizome or root size; enhanced flower size and/or colour intensity; increased biomass; larger and/or thicker leaves; dwarfism (Hannweg and Vos 2001). The results found in the treatment of *A. maemsii* (Beck *et al.* 2003) further indicate that an increase in colchicine concentration and treatment exposure time influence the reduction in root length and seedling (plant) height.

According to Tilney-Bassett (1986) and Martelotto *et al.* (2005), the improvement of many agricultural and horticultural crop plants by increasing flower size and fruit and seed weight is a particularly attractive prospect. The colchicine treatment of a yellow-flowered cyclamen (*Cyclamen persicum* Mill.) influenced the tetraploid plants to have deep-yellow flowers compared to their diploid associates (Takamura and Miyajima 1996). Pollen grains of tetraploid plants are larger in diameter than diploid ones (Tilney-Bassett 1986, Cohen and Yao 1996, Takamura and Miyajima 1996).

Tilney-Bassett (1986) further reports that in *Amaranthus*, *Cammellia*, *Citrus* species and grapes, particular attention was drawn to the clear mosaic or sectoring created by the contrast between the normal green diploid tissues and the darker green tetraploid tissues. Although less important than the vegetative

growth for the initial recognition of polyploid areas, the flowers of cranberry and grapes were enlarged by polyploidy, as were the fruit. Diploid apple fruit had thicker skin than for the tetraploid.

Experiments done in yellow-flowered cyclamen (Takamura and Miyajima 1996), *Acacia mearnsii* (Beck *et al.* 2003), *Zantedeschia* cultivars (Cohen and Yao 1996) and many other plants (Tilney-Bassett 1986) show that tetraploid leaves have larger guard cell size and fewer stomata than their diploid relative. Moreover, the well-developed chloroplasts of the stomatal guard cells increase with increasing ploidy levels.

Polyploid plants may have improved photosynthetic capacity, drought tolerance, pest resistance and increased secondary metabolite production, such as medicinal compounds (Hannweg and Vos 2001, Martelotto *et al.* 2005). However, Tilney-Bassett (1986) reports that polyploids frequently have disadvantages initially, for example, the reduction in resistance to drought and cold and lower fertility and yield. These can probably be overcome by further breeding and selection at the polyploid level.

Tilney-Bassett (1986) reports that in the pineapple, polyploids flowered later, fruit maturity was delayed, and overall fruit size reduced even though the individual fruitlets (or eyes) were larger. Initially, pineapples had a lower sugar content, a higher percentage of water, and a lower dry matter content in the polyploid than the corresponding diploid variety. Clearly, there was a need for much further improvement through breeding and selection before these polyploids could be of commercial value. Tetraploid apple trees were shown to be vigorous growers compared to the diploid parents.

According to Gao *et al.* (1996), the tetraploid plants of *Datura stramonium* contained 1-2 times higher alkaloid content in the leaf, stem and roots compared with that in the diploid plants. The content of alkaloid in the tetraploid plant of

Atropa belladonna was 153.6% of that in the diploid plant. *Salvia miltiorrhiza* roots are used as a major traditional Chinese medicine for cardio-vascular diseases. Induced polyploid roots have a higher content of tanshinones than the diploid.

Brutovská *et al.* (1998) report that *Hypericum perforatum* L. is a medicinal plant producing hypericin (a secondary metabolite with phamarceutical effect) in leaves, stems, sepals, petals and stamens. An increased variability of several morphological characteristics and an increase in hypericin content was observed to depend on the increase in ploidy level. Thus polyploid plants had higher hypericin contents compared to their relative diploid plants.

Polyploid plants show low yield in their initial stages of growth (Tilney-Bassett 1986) but later after selection they have an increase in biomass (Hannweg and Vos 2001, Martelotto *et al.* 2005). As polyploidy can facilitate dwarfism, most dwarf plants have high fruit yield since photosynthetic products are mostly used for fruit production instead of being channelled into shoots for vegetative growth.

Polyploidy lowers plant fertility especially in its initial stages (Tilney-Bassett 1986) and usually enhances apomixis (asexual seed production) (Martelotto *et al.* 2005). Reproduction studies have shown that about 97% of *Hypericum perforatum* L. polyploid seeds developed apomictically (apospory with pseudogamy) (Brutovská *et al.* 1998). Yellow-flowered Cyclamen tetraploids had a slightly lower pollen viability than the diploid, but they could easily be self-pollinated and/or crossed with other tetraploid cultivars (Takamura and Miyajima 1996). All polyploid *Salvia miltiorrhiza* plants were semi-sterile compared to diploid (Gao *et al.* 1996).

Snyder *et al.* (1985) report that polyploid plants occurring in nature almost always have an even number of sets of chromosomes because organisms having an odd number have low fertility. Organisms with three complete sets of

chromosomes are known as triploids. As far as growth is concerned, a triploid will be quite normal because the triploid condition does not interfere with mitosis; in mitosis in triploids (or any other type of polyploid), each chromosome replicates and divides just as in a diploid. However, because each chromosome has more than one pairing partner, chromosome segregation is severely upset during meiosis and most gametes are defective. Unless the plant can perpetuate itself by means of asexual reproduction, it will eventually become extinct.

According to Snyder *et al.* (1985), the infertility of triploids is sometimes of commercial benefit. For example, the seeds in commercial bananas are small and edible because the plant is triploid and most of the seeds fail to develop to full size. Compton *et al.* (2000) report that seedless watermelons are highly prized by consumers because they are sweeter than seeded diploid cultivars and lack hard seeds. However, seedless melons are rare in the market due to difficulties associated with obtaining new tetraploid breeding lines and poor germination of triploid seed.

Snyder *et al.* (1985) further report that tetraploid organisms can be produced in several ways. The simplest mechanism is failure of chromosome separation during mitosis, which instantly doubles the chromosome number. In a plant species that can undergo self-fertilization, such an occurrence creates a new, genetically stable species because the chromosomes in tetraploids can pair in two by two during meiosis and therefore segregate regularly, each gamete receiving a full diploid set of chromosomes. Self-fertilization of the triploid restores the chromosome number, so the tetraploid condition can be perpetuated.

CHAPTER THREE

Material and Methods

3.1 Material

Plant material for identification and preparation of the herbarium specimens and seeds for germination and ploidy studies were collected in the veld. The area of collection included the North Coast region of KwaZulu-Natal, specifically areas close to Mtunzini, Empangeni, KwaDlangezwa, Esikhawini, Richards Bay, Mtubatuba, Enseleni, Eshowe and Melmoth (Nkandla) (Figure 3.1).

Figure 3.1: Map showing the areas for species collection

3.2 Method

3.2.1 Specimen and seed collection and preparation

3.2.1.1 Specimen collection, pressing, drying and mounting

Collecting, pressing, drying and mounting methods were in accordance with Fish (1999). Diversions from these methods due to the peculiar growth form of the selected species are discussed later.

The Cucurbitaceae has male and female flowers occurring on the same or on separate plants and flower collection took this into consideration. Collected fruits were sectioned longitudinally and across. This is also a diagnostic feature important for identification of these species.

Cucurbitaceae flowers are delicate and short-lived and they were therefore placed separately in wax or tissue paper for pressing. Small, gummed cards were used, especially where the pressed flowers had to be dissected. The fruits of these species appeared to be drying too slowly and so the press was put in a deep freezer for one or two days and then returned to the drier.

To prevent insect attacks on pressed material, specimens were placed in a deep freezer for a minimum of 72 hours. Wiping the pressed fruit with absolute ethanol (99% v/v) and changing the flimsy (specimen holder) prevented fungal attacks on pressed fruits. After the alcohol had dried, the specimen was returned to the drier.

3.2.1.2 Species identification

Taxonomic and simple keys (field guides) were used for identifying collected specimens. Closely related specimens were identified by means of a taxonomic key. To confirm identification of collected material, herbarium specimens were used. Mounted, identified specimens are housed in the University of Zululand (ZULU) and KwaZulu-Natal (KNH) Herbaria collections.

3.2.1.3 Seed collection, drying and storage

The seeds were collected from the same plant communities where the herbarium specimens were obtained. The period of collection varied depending on the time of seed ripening and maturation. Mature hard-rind fruits of *Lagenaria sphaerica* could be kept in the laboratory for longer periods before seed removal from the fruits but seed removal from the soft-rinded fruits of *Coccinia palmata* had to be carried out immediately due to fruit decomposition.

Seeds were placed on paper and sun-dried. The paper absorbed the mucous covering the seeds, thereby eliminating fungal growth during the seed drying period. The seeds were stored in sealed plastic containers at room temperature.

3.2.1.4 Sterilization

a) Specimens

Identified or unidentified, mounted or non-mounted specimens were placed in a deep freezer for a minimum of 72 hours to kill all the insects that feed on the plant material.

b) Seeds

Seeds were soaked in 1% (v/v) of 3.5% m/v sodium hypochlorite (Reckitt Benkisor™) for 20 min for surface sterilization and rinsed three times with sterile water under sterile conditions.

c) Equipment

Liquid substances (water, colchicine, etc.) and other equipment (forceps, Petri dishes, etc.) were sterilized at 120^{0}C for 20 min using a Speedy Autoclave (vertical type).

3.2.2 Plant usage by local communities

Communities from KwaDlangezwa to Richards Bay were interviewed since *Lagenaria sphaerica* occurs frequently in this region. Eshowe and Nkandla communities were included to establish the uses of *Coccinia palmata* which occurs mostly in that region. A questionnaire was used to extract information from respondents (Appendix 1).

3.2.3 Germination

a) Fresh and dry weight and percent moisture content

Fresh weight and changes of fresh and dry weight during germination were determined based on five replicates. The average of the five replicates was calculated. Each replicate consisted of five seeds because of the small size and low number of seeds available.

Initial fresh weight was determined for each seed batch, followed by daily changes in fresh weight. Dry weight was determined after seeds were dried at

100°C for 24 hours. Water content as a percentage was calculated according to the formula given by the International Seed Testing Association (ISTA) (Bewley and Black, 1985):

$$\% \text{ Moisture content} = \frac{\text{Fresh weight of seed} - \text{dry weight of seed}}{\text{Fresh weight of seed}} \times 100$$

b) Optimum germination conditions

The seeds were germinated at different temperatures (15°C, 20°C, 25°C, 30°C and 35°C) under both light (in growth cabinets with 6 x 11W fluorescent tubes) and dark conditions to determine the best germination conditions.

c) Seed viability

The effect of aging on seed viability was tested by germinating seeds of different ages under identical optimal conditions.

d) Germination procedure

Sterilized seeds (20 seeds per 12.5 cm Petri dish) were transferred under sterile conditions into 10 ml sterile water in Petri dishes lined with filter paper. To reduce evaporation, the Petri dishes were sealed with masking tape or parafilm.

e) Polyploidization

Colchicine was used to induce polyploidy in seeds. The seeds were treated with colchicine either before or after seed incubation for germination. Seeds were incubated under light conditions at 25°C. For each treatment, five replicates of 20 sterilized seeds each were treated in 10 ml colchicine with different concentrations ranging between 0.001g/L (v/v) and 0.1g/L (v/v). After treatment

with colchicine, seeds were rinsed in sterile distilled water and incubated in 10 ml sterile distilled water.

Twenty seeds were incubated in 12.5 cm Petri dishes sealed with masking tape. Seeds were colchicine-treated for one, three and ten days before incubation. The seeds were again treated with colchicine for one and three days after three, six and nine days' incubation for germination.

3.2.4 Seedlings

a) Establishment

Seedlings with a well-developed plumule and green cotyledons (after growing for approximately 12-18 days) were transferred to the seedling trays and were hardened at <300 µmol/m^2/s light intensity (80% shade) in the nursery.

b) Development and growth

i) Diploid

Seedlings with a minimum of three developed leaves (after 36-40 days / 5-6 weeks) were transferred to pots filled with a riversand-vermiculite mixture (1:1). They were finally grown under the following light intensities: <300 µmol/m^2/s (80% shade); 400 µmol/m^2/s (70% shade); 600 µmol/m^2/s (40% shade); 800 µmol/m^2/s (30% shade) and >1000 µmol/m^2/s light intensity (0% shade).

ii) Polyploid

Two experiments for seedling growth were performed. For the first experiment, well-hardened seedlings in pots (5-7 seedlings per 20l pot) were grown at <300 µmol/m^2/s (80% shade) and 400 µmol/m^2/s light intensity (70% shade) in the

nursery. Soon after transfer to the pots, seedlings were irrigated daily for three weeks and then once every two days. Plants were fertilized with 20g of 2:3:2(22) fertilizer per pot at least once every two months.

For the second experiment, seedlings were transplanted in black plastic plant bags (1 seedling per 10 L bag) containing a 1:1 mixture of river sand and vermiculite and kept at <300 µmol/m^2/s (80% shade). On day nought, week four, week eight and week 12, plants were moved to 800 µmol/m^2/s (30% shade) and >1000 µmol/m^2/s light intensity (0% shade) in the nursery and then to the soil (>1000 µmol/m^2/s light intensity, 0% shade). Plants were planted within and without a bag (10 L) in the soil in order to investigate the pot (bag) effect on plant growth. Plants for a single treatment were spaced 2.4 m apart. Plants for different treatments were spaced 3.6 m apart. Plants were planted in holes that were initially filled up with water and left to drain. Plants planted without bags were transferred with the river sand-vermiculite mixture intact. The plants that were transplanted within their bags were covered with soil in such a manner that the bags were exposed above the soil surface. Regular daily irrigation for one week was followed by irrigation every second day for two weeks. Afterwards, plants were irrigated only when necessary.

3.2.5 Flow cytometry analysis of leaves and roots

A small piece from a leaf or root was homogenized in 400 µL (microlitre) of Partec – Nucleic Extraction: Solution A. The homogenized cells were micropipetted into an analyzing cuvette through a microfilter followed by 1500 µl of Partec –Cystain UV Precise P: Staining Buffer. The ploidy level of the plant material was analysed using the Partec Chromosome Analyser (Partec Flow Cytometer).

Control plant material was used to set the analyser on the 100 x-axis for diploid cells and thus treated tetraploid and octoploid material should be represented at 200 and 400, respectively.

3.2.6 Morphological analysis

- Plant height was measured in millimetres (mm) at 4-6 weeks intervals.
- The number of stems and leaves per plant was counted at similar intervals.
- Leaf area was determined by means of a Delta-T area meter. For measurement, mature leaves were selected from leaves in position 8-10 from the apex.
- Stomatal counts and guard cell measurements were based on images obtained from clear, dried nail polish applied to lower leaf surfaces of growing plants. Plants are hypostomatic with stomata occurring only on the lower surface. Images were made from areas on either side of the midrib (main vein) and stomata in 10 microscopic fields (16 x 10 magnification) were measured.
- Guard cell measurements were based on images taken between 9h00-10h30 when leaves were fully turgid. A calibrated eye piece at 40 x 5 magnification was used for measurements.

3.2.7 Plant physiology

a) Fresh and dry weight and percentage moisture content

The fresh and dry weight in grams for shoots and roots were determined. For dry weight determination, plant material was oven dried at 90^0C for 24 hours or until completely dry. Variation in the drying period was needed for roots (*C. palmata*) and fruits (*C. palmata* and *L. sphaerica*) since they had higher moisture contents and were more bulky compared to the leaves.

For the progressive fresh and dry weight determination, three plants per treatment were used for each measurement as this was a destructive method and the number of plants was limited. At harvest, a minimum of 20 plants was used.

The percent moisture content for shoots, as well as roots was determined using the method prescribed by International Seed Testing Association according to Bewley and Black (1985:93): see section 3.2.3(a).

b) Chlorophyll analysis using 90% Acetone

According to Hewitson (2004), approximately 1 cm^2 of fresh leaf is cut and weighed for chlorophyll extraction, but for this investigation, a 15 mm diameter leaf or cork borer was used to cut leaf discs, which approximated the tissue requirements described by Hewitson (2004). The removed leaf piece was immersed in 5 cm^3 of 90% (v/v) acetone in a snap cap test tube and refrigerated overnight (about 14-15 hours).

Glass cuvettes were used for spectrophotometric/colourimetric readings since the plastic material is rapidly deteriorated by acetone. Absorbance (A) of the solution was read at 645 nm and 663 nm and the measurements were substituted in the following equation:

Concentration of chlorophyll extract (in mg/cm^3) = (20.2 x (A645 nm)) + (8.02 x (A663 nm)).

To eliminate the error of unextracted chlorophyll in the leaf discs, the obtained leaf discs were ground with a mortar and pestle, and filtered instead of being stored in the fridge.

c) Chlorophyll analysis using Dimethyl Sulphoxide/Sulfoxide (DMSO)

According to Richardson *et al.* (2002), chlorophyll extraction using dimethyl sulphoxide (DMSO) has two principal advantages over other extractions such as methanol, ethanol or acetone. Firstly, the method is faster, largely because grinding and centrifuging is not required. Secondly, the chlorophyll extracts are more stable in DMSO, and do not break down as quickly as those in acetone. Further, DMSO extracts are stable for up to five days, whereas with acetone extracts the measured level of chlorophyll begins to decrease immediately.

For the extractions, glass test tubes containing 7ml DMSO were preheated to 65^0C in a water bath. Chlorophyll was extracted from seven leaf discs, from each leaf sample (approximately 100mg (FW) for all seven leaf discs).

Chlorophyll extraction was completed or terminated at maximum of 50 minutes. Even after 50 minutes some discs still had green patches, showing that the chlorophyll was not fully extracted. According to the method illustrated by Richardson *et al.* (2002), there is no loss of chlorophyll in the heated DMSO during the first hour. Chlorophyll extraction was therefore allowed for the maximum of 50 minutes.

When the chlorophyll extractions were complete, samples were removed from the water bath and each vial or test tube was topped up to exactly 10ml with DMSO using a Pasteur pipette. Pure DMSO was used as a blank to calibrate the spectrophotometer. Absorbance of both blank and sample were measured at 645 and 663nm.

Richardson *et al.* (2002) demonstrated that the absorption spectrum (600-680nm) for chlorophyll extracted in DMSO was virtually identical to that extracted in 90% acetone. The equations used are as follows: Chla (g/l) = 0.0127 A_{663} –

0.00269 A_{645}; Chlb (g/l) = 0.0229 A_{645} – 0.00468 A_{663}; and tot Chl (g/l) = 0.0202 A_{645} + 0.00802 A_{663}. For research purposes, the total chlorophyll (g/l) was determined for all species and the final answer was converted to mg/cm^3. The chlorophyll concentration of the extract calculated from these equations was then converted to leaf chlorophyll contents (mg Chl cm^{-2} leaf area).

3.2.8 Phenological analysis

The periods from seed germination (day 0) to the emergence of the first leaf, emergence of the first flower, the first fruit, and the plant growth declining stage, were measured in terms of days and months.

CHAPTER FOUR

Results and Discussion

4.1 Species identification

About 50 specimens of Cucurbitaceae were collected, identified and prepared for herbarium purposes. Nine genera and eleven species were identified (Table 4.1). The main areas where material was collected are shown in the table, but more precise localities appear on the labels of the herbarium specimens. The Cucurbitaceae species occur mainly in dispersed groups or in clumps of communities. The majority is found in forest margins and amongst thick shrubs (thickets) and a few also occur in grasslands. Their growth conditions range from shade to full sun. The whole plant or only parts of a plant may grow in shady conditions. Only one exotic species, *Luffa cylindrica* (L.) Roem, was collected. It is very rare and it has a high commercial value e.g in the production of bath sponges, engine filters and shock absorbers.

Herbarium specimens of *Cucumis metuliferus* E. Mey. ex Naud. were prepared from plant material grown at Unizul Campus from seeds collected at Ulundi and Nelspruit. *C. palmata* and *L. sphaerica* were observed in protected areas such as Imvubu Lodge at Richards Bay but were not collected.

The identified species served as the basis from which certain species were selected for germination, polyploidy induction and growth studies. Parameters used for selection were the importance of the species for food and medicinal purposes and sufficient seed availability. As a result *C. palmata* (Sond.) Cogn., *L. sphaerica* (Sond.) Naud., *M. balsamina* L. and *M. foetida* Schumach. were selected for the initial germination study.

Table 4.1 Cucurbitaceae species identified in the collecting area

Species name	Common name	Locality	Community	Light
Coccinia palmata (Sond.) Cogn.	Wild cucumber	Eshowe (Ntumeni)	forest margin	partial shade
		Nkandla Forest Reserve	forest and forest margin	full to partial shade
		Empangeni	forest and forest margin	full to partial shade
Coccinia rehmannii Cogn.	Wild cucumber	Mtubatuba	grassland or lawn	full sun
		Ndumo (Emboza)	forest	partial shade
		Nkandla (KwaNxamalala)	thicket and grassland	partial shade and full sun
Coccinia species		Nkandla Forest Reserve	forest	partial shade
Cucumis metuliferus E. Mey. ex Naud.	African horned cucumber, Spiny cucumber	Nelspruit	thicket	partial shade
		Ulundi	forest	full shade
Lagenaria sphaerica (Sond.) Naud.	Wild melon	Eshowe (Ntumeni)	thicket and grassland	partial shade and full sun
		KwaDlangezwa (Cross roads)	thicket	partial shade
		Nkandla (Town vicinity)	grassland	full sun
		Esikhawini (Madlankala, Mkhobosa, Mpembeni)	thicket and forest margin	partial shade
		Richards Bay (Mzingazi and Hillside Vicinity)	forest margin and grassland	partial shade and full sun
*Luffa cylindrica** (L.) Roem.	Sponge or smooth gourd	Mtubatuba	cultivated* (hanging on forest fence)	full sun
Momordica balsamina L.	Balsam pear or apple, African cucumber	KwaDlangezwa (Matholonjeni, Unizul Campus)	thicket and forest margin	full sun and partial shade
		Mtunzini (Twinstreams)	forest margin	partial shade
		Richards Bay Minerals	grassland	full sun

Table 4.1 Cucurbitaceae species identified in the collecting area (continued)

Species name	Common name	Locality	Community	Light
Momordica foetida Schumach.		Eshowe (College vicinity, Ntumeni, Umlalazi)	thicket and grassland	partial shade and full sun
		KwaDlangezwa (Unizul Campus)	thicket	partial shade
		Nkandla (Mooiplaas)	grassland	full sun
Mukia maderaspartana (L.) Roem.		Enseleni	grassland	full sun
		Eshowe (Ndikilini)	grassland	full sun
		KwaDlangezwa (Unizul)	closed woodland	shade
		Mtunzini (Twinstreams)	forest margin	partial shade
		Owen Sithole College of Agriculture	forest margin (hanging on forest fence)	partial shade
		Ubisana Valley	grassland	full sun
Peponium mackenii (Naudin) Engl.		Eshowe (Ntumeni)	cultivated sugarcane field	partial shade to full sun
		Nkandla Forest Reserve	forest	partial to full shade
Zehneria scabra (L.f.) Sond. (subsp.) *scabra*		Eshowe (Nkwalini)	thicket	partial shade to full sun
		KwaDlangezwa (Unizul Campus)	grassland and forest margin	partial shade to full sun
		Nkandla Forest Reserve	forest margin	partial shade

Legend: * : not indigenous

Figure 4.1a: *Lagenaria sphaerica* (Sond.) Naud. plant showing male flowers (Pooley 1998)

Figure 4.1b: Fruit of *L. sphaerica* (Welman 2004)

Figure 4.2: *Coccinia palmata* (Sond.) Cogn. plant (Pooley 1998)

4.2 Utilization of *C. palmata* and *L. sphaerica* by the communities

The communities where *C. palmata* (Figure 4.2) and *L. sphaerica* (Figures 4.1a and b) were collected were the target areas for the determination of usage of these species. *C. palmata* was mainly collected from Eshowe (Ntumeni) and Nkandla Forest Reserve while *L. sphaerica* was collected from Eshowe, Empangeni and Richards Bay. Since *L. sphaerica* is widely distributed and there were few *C. palmata* plants observed in the Esikhawini–Richards Bay area, questionnaires for both species were determined simultaneously in all areas of collection. The areas surrounding the main area of species collection were then used for interviewing purposes, for example, Eshowe (Ntumeni), NKandla (Nkandla Forest Reserve or Esibhudeni, Bhamu or KwaShange, Entembeni and KwaNxamalala), Empangeni (KwaDlangezwa and Esikhawini) and Richards Bay (Mandlazini, Mzingazi, KwaMbonambi, KwaSokhulu).

People were interviewed either individually or in a group. In the case where a group of people or a household was interviewed and the same response was recorded, the group or household was recorded as one respondent.

4.2.1 *C. palmata*

A total of 101 respondents were interviewed of which only 22 respondents gave a positive response. Of the 22 respondents knowing the species, 19 (86%) were from Nkandla, 2 (9%) from Eshowe (Ntumeni) and 1 (5%) from Richards Bay (Mandlazini). It was noticed that interviewees from the Nkandla area were confusing *C. palmata* with *Coccinia rehmannii* Cogn. because their fruits and leaves look alike, although *C. palmata* has bigger leaves than *C. rehmannii.* As a result, the response to the questionnaires for this species was therefore not recorded since it was not reliable.

4.2.2 *L. sphaerica*

Regarding *L. sphaerica*, 155 respondents were interviewed and only 47 knew the species (i.e. had a positive response). Of these, 25 (53%) people were from Empangeni, 16 (34%) from Richards Bay, 5 (11%) from Eshowe and 1 (2%) from Nkandla. The gender and age of the respondents appear in Table 4.2. Table 4.2 illustrates that 70% of the respondents were females and only 30% were males.

Table 4.2: Gender and age (years) status of the respondents

Gender	Age (years)							
	15-24	25-34	35-44	45-54	55-64	65-above	Total	Total %
Male	0	3	2	4	2	3	14	30
Female	0	2	12	11	6	2	33	70

Of the 14 male interviewees, 64% fall within the age group above 45 years and only 36% in the age group below 45 years. No males in the age group 15-24 years knew the uses of, or used *L. sphaerica*.

The female interviewees with an age from 35-44 and from 45-54 had almost the same knowledge on usage of this species, with 36% and 33% of the respondents, respectively, having knowledge of usage. These age groups are followed by a group of 55-64 with percentage interviewees of 18%. The group of females below 35 years and over 65 years had a low percentage (6%) of interviewees. It was observed during the interview sessions that young people had very little or no knowledge about indigenous plants since they are more exposed to a modern life style. It was, however, very difficult to get hold of elderly people over 65 years, since most of them have passed away.

None of the interviewees knew the English common name for *L. sphaerica*. In addition to what is known from the literature, the following (Zulu) names were also used by the respondents interviewed: *uswela lwentaba, uswela lwasendle, uswela lwehlathi, uswela lwe(zi)nyoka, uthanga lwe(zi)nyoka, ithanga le(zi)nyoka, umnungu mabele* and *umazimilele*. The name "*uswela*" and "*uselwa*" was used interchangeable depending on the places. In Shangaan this species is called *isirakarakane*.

Information gained from the communities indicated that *uswela* or *uselwa* means gourd. This can be used for both *L. siceraria* and *L. sphaerica*. In order to differentiate the two species, mostly people call *L. sphaerica uswela lwehlathi, lwentaba* or *lwasendle,* meaning that it occurs in the bush or wild and it is not cultivated. "*Uthanga*" is the plant; and "*ithanga*" is the fruit of *Cucurbita maxima* (ordinary pumpkin), but then for *L. sphaerica*, a suffix of "snake(s)" is added to denote that it is only eaten by snakes and is poisonous to people, for example *u(i)thanga lwe(zi)nyoka*. The expression is also the same for *uswela lwe(zi)nyoka*. The word *uthangazane* has to do with *Cucurbita maxima* but the meaning is uncertain.

"*Uswela lwamakhosi (lwenkosi)*", meaning "gourd for king(s)", was derived from the species usage, since it was used specifically by the kings for many traditional ceremonies and also to cleanse themselves. The meaning of "*umnungu mabele*" is uncertain, while the word "*umazimilele*" means that the plants are not cultivated but just grow on their own.

The respondents indicated that plants are collected from the wild and are not cultivated. They also plant them next to their houses for convenience. Fruits, the part that is mostly used, are collected mainly in summer to autumn (September to February) when they occur abundantly. Some fruits are collected from the wild and sold in "muthi markets", for example in Durban.

L. sphaerica is used for food, medicine and other purposes, such as for instrument making. Some of the interviewees were of the opinion that only snakes and not people eat this plant, especially the fruits. Others regarded the fruits of this species as poisonous when eaten, while others regarded the vegetative parts as good cow fodder.

The results obtained from the communities show that roots, shoots and fruits are used for various medicinal purposes. Roots are used as an emetic for excessive bile. The respondents said that roots are cooked and a cupful of the extract is taken at night. The next morning, a person drinks plain water for emetic purposes. Chopped and boiled fruits are also used for emetic purposes. The resulting decoction of a quarter of a cup to one cup is drunk, and then water is immediately taken to induce regurgitation. It is reported that this species has been used predominantly by chiefs and kings as emetics and for baths before their ordinations and during their kingship and in other traditional ceremonies. A decoction made from boiling the fruit with other plants is taken to improve the immune system of HIV and AIDS patients.

The leaves of *L. sphaerica* are boiled and sieved and used as an enema for flu, especially for children and infants. It is advised that the enema be made very weak when used. The fruit is regarded as a very strong, dangerous enema and must be carefully used. One small to medium-sized fruit is chopped or crushed and either mixed with lukewarm water or boiled with about 2 litre water. The mixture is sieved and the liquid part is used as an enema for flu, back ache and scrotum elephantiasis. The prepared medicine can be stored in sealed bottles and used later for enema purposes. Kings and chiefs also used this mixture as an enema to cleanse themselves. Pregnant women, to release the abnormal positioning or growth of the foetus in the womb, can also boil the chopped fruit with sodium bicarbonate and drink the decoction.

The plant is used to treat animals such as dogs, horses and cattle. One chopped fruit is mixed with the plant called *izimbombo*, as well as sorghum, and boiled in about a liter of water. The resulting decoction is given to dogs, horses and cows as a drink when they are sick, especially when intestinal worms are suspected. Fruits are also given as medicines to cattle in order to change their colour and make them spotty. Further, it was also reported that cut fruit is rubbed against a dog's mouth to induce vomiting. This is believed to energize the dogs when they cannot hunt effectively.

The fruits were traditionally used as a musical instrument. A small hole was opened at the top of cooked mature fruit (with hard rind) to remove flesh and seeds. The rind was then tied with strings to make an instrument that resembled a guitar *(umakhweyana(e))* that was played by ladies.

The use of *L. sphaerica* fruits as an enema for adults is well known by the communities and 53% of the respondents knew about this usage. The usage of the plant for enema and ceremonial purposes is known from the literature but the parts used, as discussed in the literature, are different from the obtained results.

4.3. Germination

4.3.1 Seed Collection

Seeds were initially collected for all the species occurring in the studied area (Table 4.3). Seeds were used to study germination and polyploidy. Initial experiments indicated that *Momordica balsamina* and *M. foetida* did not germinate when subjected to laboratory testing conditions. Based on these results and the availability of seeds, *C. palmata* and *L. sphaerica* were selected for this study.

4.3.2 Factors affecting seed germination

4.3.2.1 Temperature and light

The effects of temperature and light were determined on the germination of four species: *C. palmata*, *L. sphaerica* and *M. balsamina* (9 months) and *M. foetida* (2 weeks). Seeds were germinated at 15°C, 20°C, 25°C, 30°C and 35°C. Seeds used were collected from Eshowe, except for *M. balsamina* of which seeds were from KwaDlangezwa. Five replicates of 20 seeds each were used.

All species, except *C. palmata*, had a very low germination percentage under all conditions with their highest germination being 30%. Figure 4.3 indicates that *C. palmata* seeds incubated for 16 days had high germination percentage (80%) under light conditions at temperatures of 25°C and 30°C. Although dark treatment at 25°C resulted in 70% seed germination, higher temperatures completely inhibited germination.

Table 4.3: Locality and seed collection periods of species of Cucurbitaceae family

Species name	Locality	Community	Collection period
Coccinia palmata (Sond.) Cogn.	Eshowe (Ntumeni)	Forest margin	April – June
	Nkandla Forest Reserve	Forest and forest margin	June - August
Coccinia rehmannii Cogn.	Mtubatuba	grassland	August
	Ndumo (Emboza)	Forest	June
Cucumis metuliferus E. Mey. Ex Naud.	KwaDlangezwa (Unizul)	Garden	May-August
	Nelspruit	thicket	September
	Ulundi	forest	June
Lagenaria sphaerica (Sond.) Naud.	Eshowe (Ntumeni)	thicket and grassland	April – June
	KwaDlangezwa (Cross roads)	thicket	June – November
	Esikhawini (Madlankala, Mkhobosa, Mpembeni)	thicket and forest margin	July – May
	Richards Bay (Mzingazi and Hillside Vicinity)	forest margin and grassland	August – January
*Luffa cylindrica** (L.) Roem.	Mtubatuba	cultivated (hanging on the fence)	August
Momordica balsamina L.	KwaDlangezwa (Matholonjeni, Unizul Campus)	thicket and forest margin	August – June (year round)
Momordica foetida Schumach.	Eshowe (College vicinity, Ntumeni, Umlalazi)	thicket and grassland	September – May (year round)
	KwaDlangezwa (Unizul Campus)	thicket	August – June (year round)
Mukia maderaspartana (L.) Roem.	Enseleni	grassland	June – October
	Owen Sithole College of Agriculture	forest margin	June - January
Peponium mackenii	Eshowe (Ntumeni)	cultivated sugarcane field	May

Legend: * : not indigenous

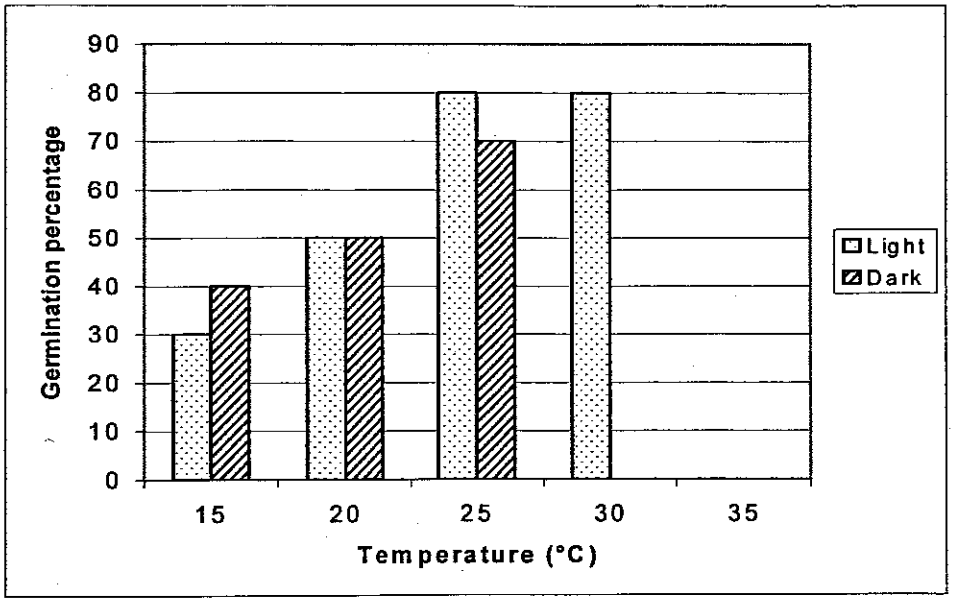


Figure 4.3: Effect of temperature and light on germination of *C. palmata* seeds from Eshowe, at 16 days' incubation

4.3.2.2 Effect of seed locality

The effect of locality on seed germination was determined on *L. sphaerica* seeds from Eshowe (9 months) and KwaDlangezwa (2 months). Five replicates of 20 seeds per Petri dish were used. Results represented in Figure 4.4 show germination of seeds from Eshowe at 8 days' incubation at all temperatures indicated and seeds from KwaDlangezwa at 6 days' incubation at 25°C and 30°C.

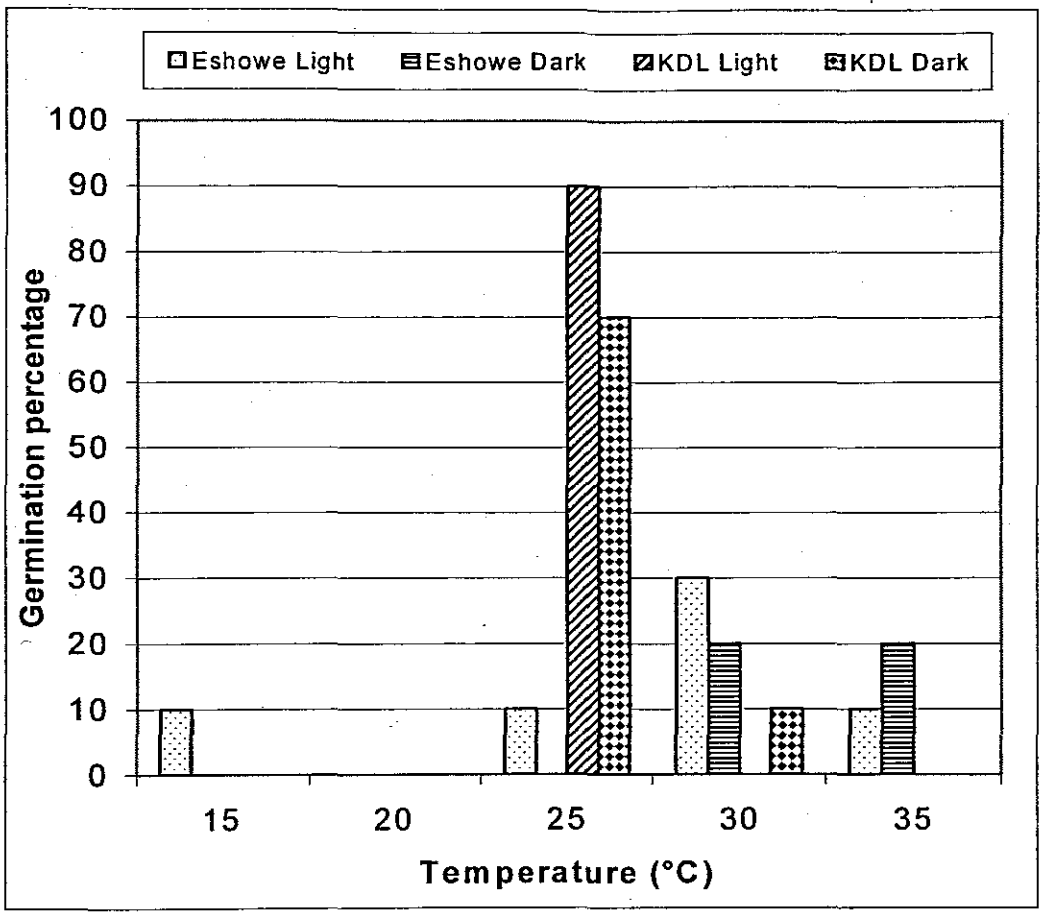


Figure 4.4: Comparison of *L. sphaerica* germination of seeds from different localities under different light and temperature conditions

Legend: KDL: KwaDlangezwa

It is clear from Figure 4.4 that *L. sphaerica* seeds collected from Eshowe have a very low germination percentage compared to seeds from KwaDlangezwa. This figure further indicates that seed from KwaDlangezwa incubated at 25°C had high germination under light (90%) and dark (70%). From the results obtained, seed incubation at 25°C under light was chosen as the best germination condition.

Confirmation of the chosen seed germination condition and locality was investigated on *L. sphaerica* seeds collected at Eshowe (Ntumeni) in mature (E-M) and immature (E-I) fruits compared with seeds collected at KwaDlangezwa Cross Roads from mature (K-M) and ripe (with dry fruit rind) (K-R) fruits. The mature seeds are collected from the fruits with yellow pulp but green rind; ripe seeds are from fruits with dry pulp and brown rind. The *C. palmata* seeds collected from Eshowe were also included.

Figure 4.5 illustrates that *C. palmata* seeds maintain high germination percentage (80%) at these germination conditions. It is clear from this figure that seeds collected from Eshowe had a significantly lower germination percentage (lower than 20%) compared to KwaDlangezwa collected seeds (70-90%). Of the seeds collected from KwaDlangezwa, mature seeds which had the highest germination percentage (90%) were selected to continue with the research.

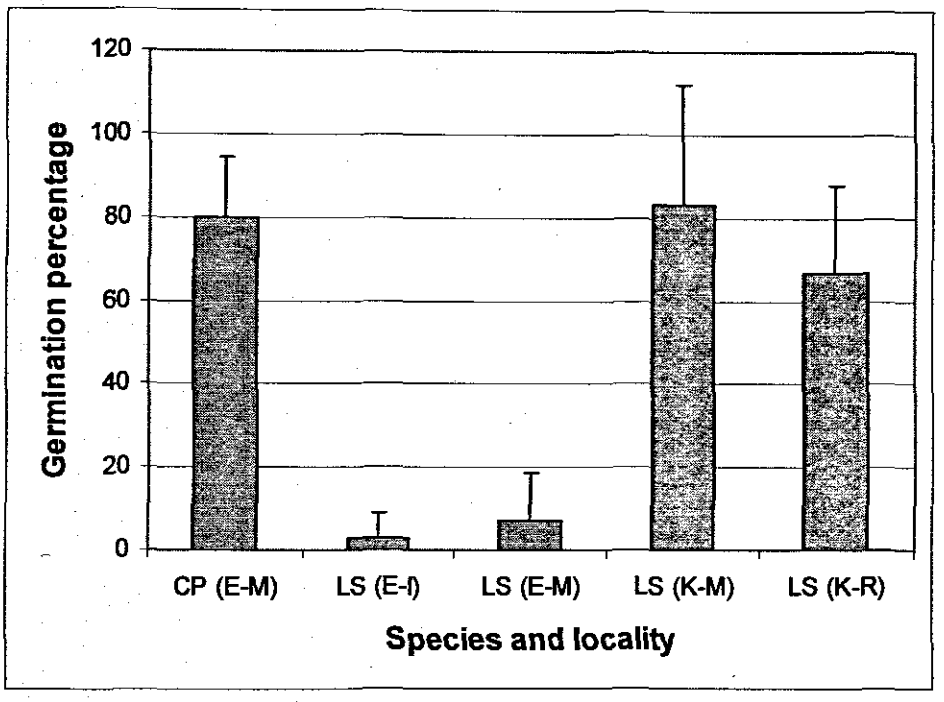


Figure 4.5: Effect of locality on germination of *C. palmata* and *L. sphaerica* seeds incubated in light at 25°C.

Legend: CP: *C. palmata*; LS: *L. sphaerica*; E: Eshowe; E-I: seeds from immature fruits from Eshowe; E-M: seeds from mature fruits from Eshowe; K-M: seeds from mature fruits from KwaDlangezwa; K-R: seeds from ripe fruits from KwaDlangezwa.

4.3.2.3 Seed age

Seed age refers to the period that elapsed between seed collection and initiation of incubation. The effect of seed age on the germination of *C. palmata* and *L. sphaerica* was determined. *C. palmata* seeds used were eight and ten months old (Nkandla Forest Reserve) and nine, ten and eleven months old (Eshowe). *L. sphaerica* seeds were three, four, 24 and 26 months old (KwaDlangezwa Cross Roads).

Initially, five replicates of 20 seeds per Petri dish were used for both species due to limited quantity of seed. At a later stage, replicates were increased up to 16 for

L. sphaerica (at 24 and 26 months) because of highly reduced seed germination percentage observed on the seed germination test run.

Results, as represented in Figure 4.6, indicate that three and four month-old *L. sphaerica* seeds germinated significantly better than 24 and 26 month-old seeds. *C. palmata* seeds from Eshowe germinated better than seeds from Nkandla at the same age of 10 months, though the difference is not significant.

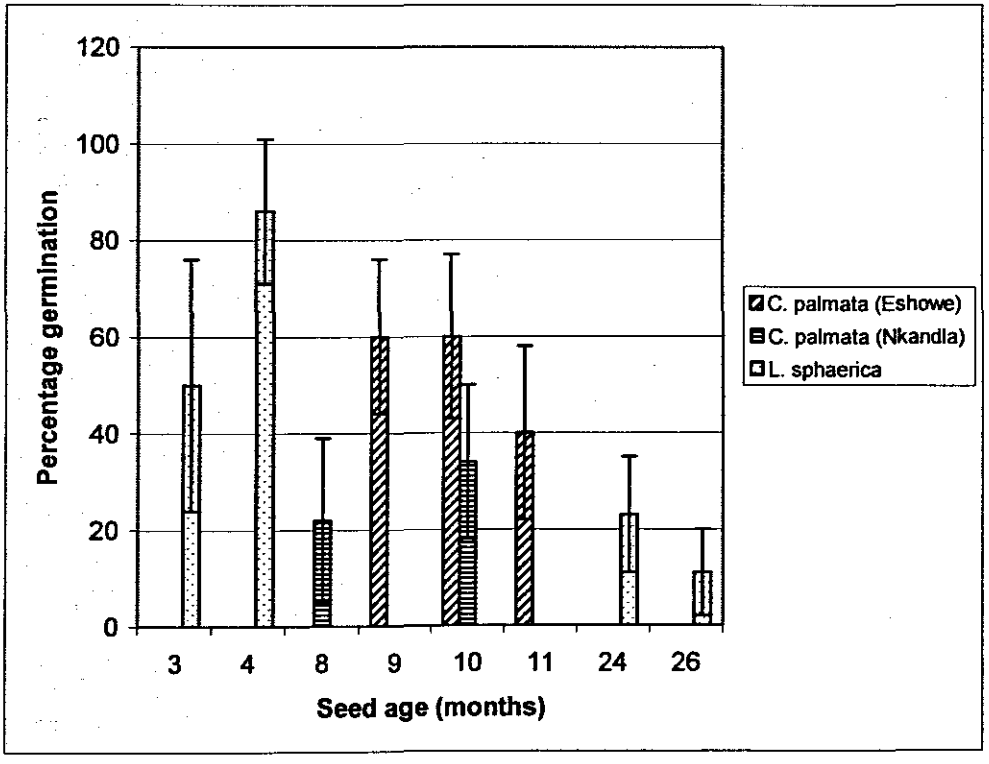


Figure 4.6: Effect of seed age and locality on germination of *C. palmata* and *L. sphaerica* seeds at seven days' incubation

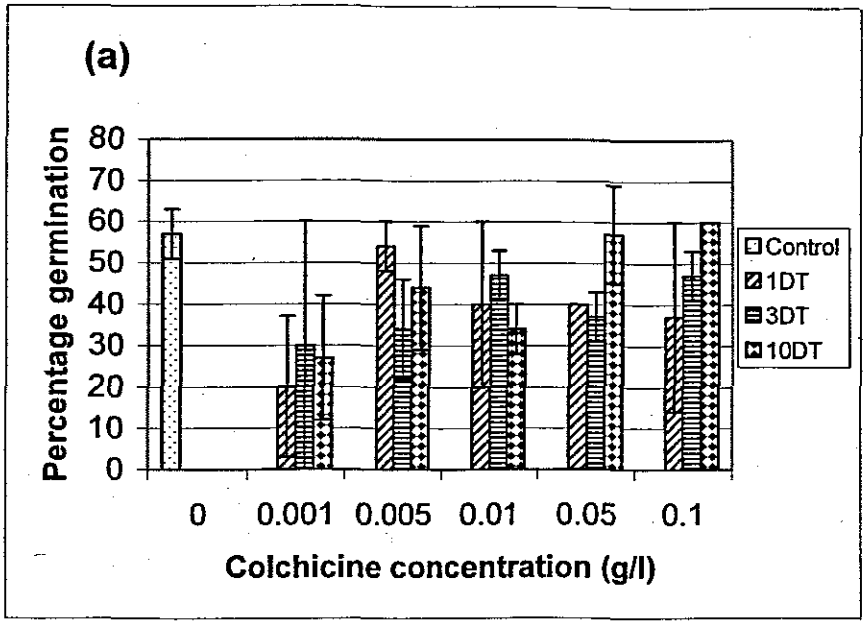
4.3.2.4 Colchicine and age effects on seed germination

The effect of colchicine treatment and seed age on germination were investigated on *C. palmata* and *L. sphaerica* seeds. *L. sphaerica* seeds were collected from KwaDlangezwa Cross Roads and *C. palmata* seeds from Eshowe. Seeds were treated with different colchicine concentrations (see section 3.2.4(e)). Seeds were treated with colchicine before and after incubation prior to germination.

a) Colchicine treatment before incubation for germination

Different colchicine concentration treatments were followed by incubation under light at 25°C. *L. sphaerica* seeds were five months old and *C. palmata* seeds were 12 months. Three replicates per treatment of 20 seeds each were tested. Treatments were one, three and 10 days with colchicine concentrations ranging from 0.001 to 0.1 g/L. The results reflect germination after six and eight days after incubation.

Table 4.4 (Appendix II) and Figure 4.7(a & b) indicate a decrease in percentage germination of colchicine treated seeds regardless of the exposure time to colchicine and colchicine concentration. However, there are minor cases, as indicated in Figure 4.7(a) where the ten day treatment with colchicine (10DT) resulted in a slight increase in percentage germination for 0.1 g/L colchicine (60%) while 0.05 g/L had no effect (57%). Treatment with 0.001 g/L colchicine had the largest effect on germination and then higher concentrations (0.05 and 0.1 g/L) had the smallest effect but seedling development was seriously affected. In general, it was observed that seedlings treated for long exposure times (more than one day) and at higher colchicine concentrations had very short radicles that sometimes rotted soon after their emergence.



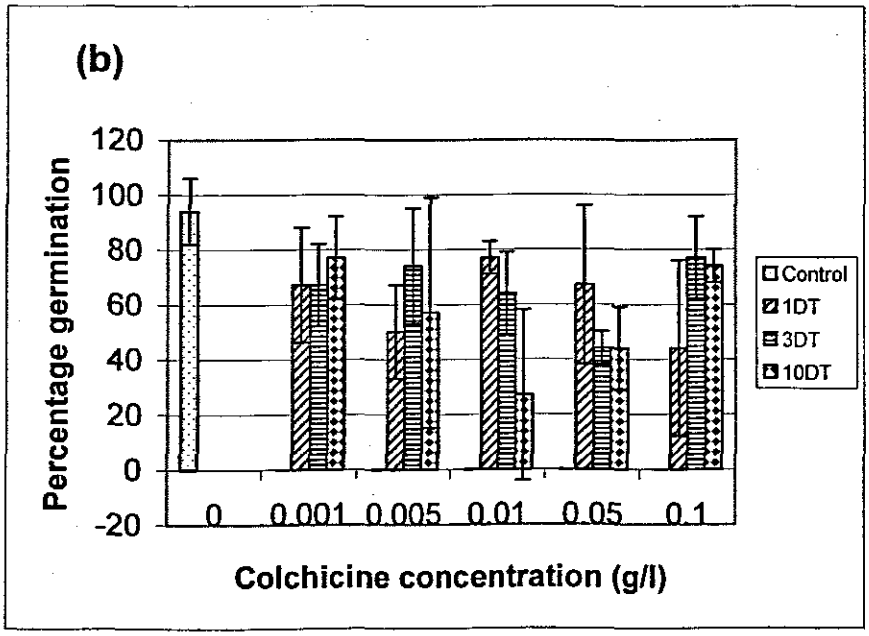


Figure 4.7. Effect of colchicine treatment before incubation on germination of *C. palmata* (a) and *L. sphaerica* (b) seeds at eight days incubation

Legend: 1 day (1DT), 3 days (3DT) and 10 days (10DT) treatment before incubation for germination

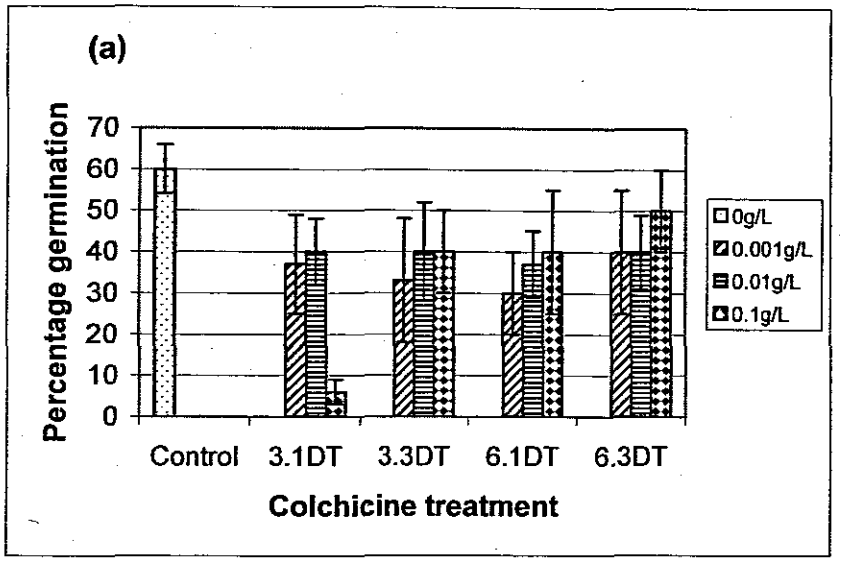
One day treatment (1DT) before incubation of 0.001; 0.01 and 0.1 g/L colchicine was selected to continue with the investigation.

b) Colchicine treatment after incubation for germination

Seeds from the same collection as for previous investigation in colchicine treatment before incubation for germination were used. Seeds were incubated for germination for three and six days, and treated for one and three days. Seeds were treated with 0.001; 0.01 and 0.1 g/L colchicine concentration. Three replicates of 20 seeds each were tested.

After 14 days incubation, as indicated in Table 4.5 (Appendix II) and Figure 4.8 (a & b) colchicine treatment causes lowering of germination percentage for both species in all concentrations, except one case for *L. sphaerica* (6.3DT; 0.01 g/L). Seeds (treatment after three days) or seeds-seedlings mixture (treatment after six days) treated for more than a day had abnormal seedling growth. Those where treatment was carried out at seed stage had seedlings with short radicles that rotted soon after their emergence. Those treated at their seedling stage had softened and decayed plumules. Treatment with 0.01 g/L colchicine concentration maintains high germination percentage in all treatments for both species (Figure 4.8 a & b).

According to the results obtained, one day treatment after three days' incubation for germination, at all colchicine concentrations, and six days' incubation for germination, at 0.01 g/L concentration, were selected to continue with the study.



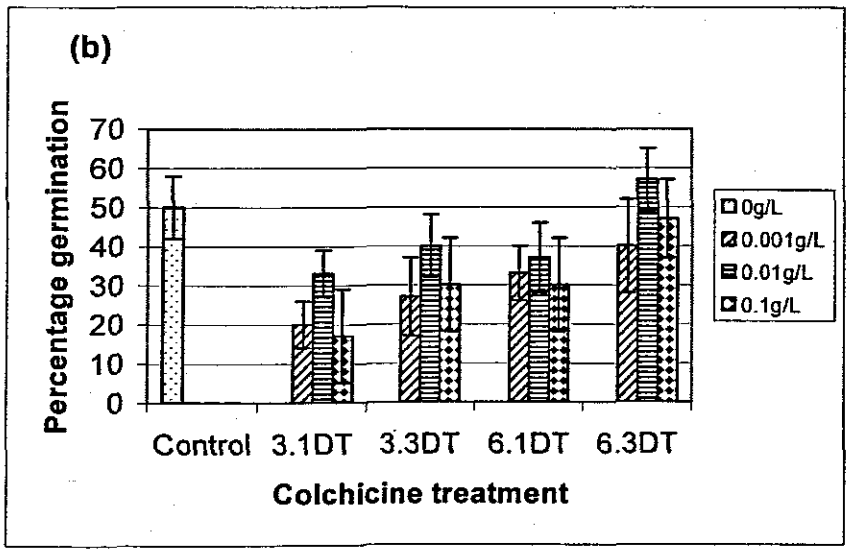


Figure 4.8: Effect of colchicine treatment after incubation initiation on germination of *C. palmata* (a) and *L. sphaerica* (b) seeds at 14 days incubation

Legend: One day treatment after three (3.1DT), six (6.1DT) and nine (9.1DT) days incubation for germination. Three days treatment after three (3.3DT), six (6.3DT) and nine (9.3DT) days incubation for germination.

c) One day treatment before (1DT) and after three (3.1DT) and six (6.1DT) days of incubation for germination, at different colchicine concentrations

As per the previous selection, 0.001; 0.01 and 0.1 g/L colchicine concentration was used for one day seed treatment before (1DT) and after three (3DT) days incubation for germination, while only 0.01 g/L was used for one day treatment after six (6.1DT) days incubation. *C. palmata* seeds were 18 months old and *L. sphaerica* were 11 months old. The results shown on Table 4.6 (Appendix II) and Figure 4.9 were recorded on day 11 of incubation.

C. palmata shows the a reduction in germination percentage of colchicine treated seeds when they are treated for one day before incubation in all concentrations. *L. sphaerica* does not show reduction except for 0.01 g/L but some colchicine treated seedlings some were bleached (the cotyledons or plumules change in texture and eventually die) before they were ready for transplantation. This can then be expressed as 13% of 0.001 g/L, 18% of 0.01 g/L and 10% of 0.1 g/L colchicine concentration treated seeds became bleached and died after their germination.

Further, Table 4.6 and Figure 4.9 show that *C. palmata* had a reduced germination percentage for 3.1DT colchicine treated seeds at all concentrations tested. *L. sphaerica*, however, showed an increase except for 0.1 g/L colchicine. At this concentration, 9% of the germinated *L. sphaerica* seeds were bleached. For 6.1DT seeds of both species, there was a reduction in germination percentage and this reduction is highly marked for *C. palmata* seeds. The 0.01g/L colchicine concentration was chosen for 1DT, 3.1DT and 6.1DT.

Table 4.6: Effect of one day treatment with different colchicine concentrations before (1DT) and after three (3.1DT) and six (6.1DT) days of incubation on *C. palmata* (18 months) and *L. sphaerica* (11 months) seed germination, at 11 days incubation

Treatment	[Colch.]g/l	*C. palmata*		*L. sphaerica*	
		% germ.	SD	% germ.	SD
0DT	0	75	9	39	7
1DT	0.001	40	9	53	4
	0.01	56	12	66	10
	0.1	46	10	9	3
3.1DT	0.001	50	13	69	12
	0.01	50	6	73	12
	0.1	50	10	34	10
6.1DT	0.01	39	7	37	8

Legend: [Colch.]: colchicine concentration in g/l

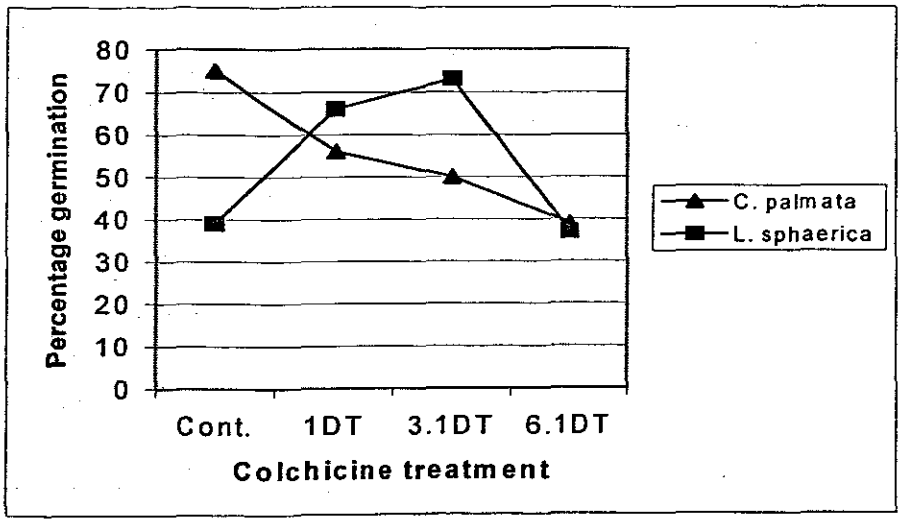


Figure 4.9: Effect of colchicine treatment before and after incubation initiation on germination of *C. palmata* and *L. sphaerica* seeds at 11 days incubation

Legend: One day treatment before (1DT) and after three (3.1DT) and six (6.1DT) days' incubation for germination.

d) One day treatment before (1DT) and after three (3.1DT) and six (6.1DT) days' incubation for germination, with 0.01g/L colchicine

C. palmata seeds (22 months) and *L. sphaerica* (1 and 14 months) were used for this investigation. The results were recorded on day 9 of incubation. Ten replicates and five replicates of 20 seeds each were tested for control and colchicine treated seeds respectively. Only 0.01 g/L colchicine concentration was used for all treatments.

According to the results shown in Table 4.7, there was no significant reduction in germination percentage of colchicine treated seeds for both species compared to their control. There was, however, a very low overall germination percentage for both species, of which the highest is less than 50%. One month-old *L. sphaerica* seeds were excluded from further investigations since they showed a very low germination percentage for both control and treated seeds. Based on the fact that seed germination for both species is initiated at a minimum of 3-5 days of incubation (see Section 4.3.2.4 and 4.2.3 (b & c)), 6.1DT was also eliminated since the treatment is essentially carried out on seedlings rather than seeds.

Table 4.7: Effect of one day treatment with 0.01g/L colchicine before (1DT) and after three (3.1DT) and six (6.1DT) days' incubation on germination

Treatment	*C. palmata*		*L. sphaerica*			
	22 months		1 month		14 months	
	% Germ.	SD	% Germ.	SD	% Germ.	SD
0DT	8	9.19	19	18.83	46	23.9
1DT	10	3.54	3	2.74	20	15.41
3.1DT	18	9.08	22	15.65	40	27.39
6.1DT	9	4.18	23	21.1	40	27.61

Legend: % Germ.: percentage germination; SD: standard deviation

e) One day treatment before and after three days' incubation for germination

C. palmata (7 months) and *L. sphaerica* (26 months) seeds were used. Sixteen replicates of 20 seeds per Petri dish were used. The increase in the number of replicates was used to overcome the problem of low germination rate that was previously recorded.

As indicated in Table 4.8 (Appendix II) and Figure 4.10, at 12 days' incubation there was a reduction in germination percentage of treated *C. palmata* seeds although not significantly, while there was an increase for *L. sphaerica*. In both species the germination percentage was very low for both treated and control seeds. There was no significant difference in germination percentage of seeds treated for one day before or after incubation, in both species.

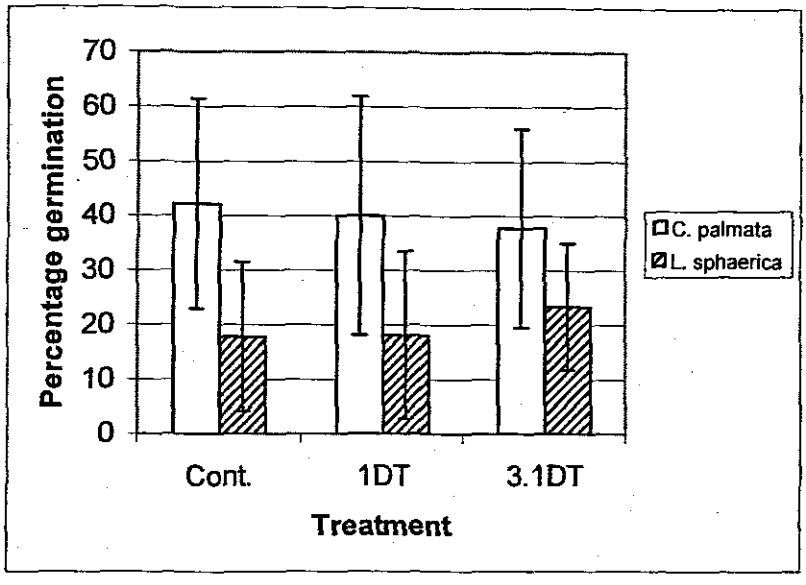


Figure 4.10: Effect of one day seed treatment before (1DT) and after three (3.1DT) days' incubation on the germination of *C. palmata* and *L. sphaerica* seeds at 12 days incubation

4.3.3 Fresh and dry weight changes and moisture content of germinating seeds of *C. palmata* and *L. sphaerica*

Dormant and germinating seeds of *C. palmata* (11 months) and *L. sphaerica* (4 months) were used to investigate the effect of germination on the seed fresh and dry weights. Fresh and dry weights of non-germinating (dormant) seeds were determined to show the moisture content of the seeds used. Three replicates of five seeds per replicate were used. The results show the average seed weight calculated for one seed.

As described in the Section 3.3.4, five replicates of 20 seeds each were used to determine seed' percent fresh weight and dry weight of germinating seeds as well as percentage germination. Even though the percentage was determined in

terms of 20 seeds per replicate, the average weight was calculated in terms of one seed.

a) Fresh and dry weight and moisture content of dormant seeds

Table 4.9 shows that there is no significant difference between fresh and dry weight of both species. The results further reflect that *L. sphaerica* seeds are 82% (4-5 times) heavier than *C. palmata* seeds in terms of fresh and dry weights. The percent moisture content of these species was 4.375% and 4.628 % for *C. palmata* and *L. sphaerica*, respectively, according to the formula derived by the International Seed Testing Association (ISTA) (Bewley and Black, 1985).

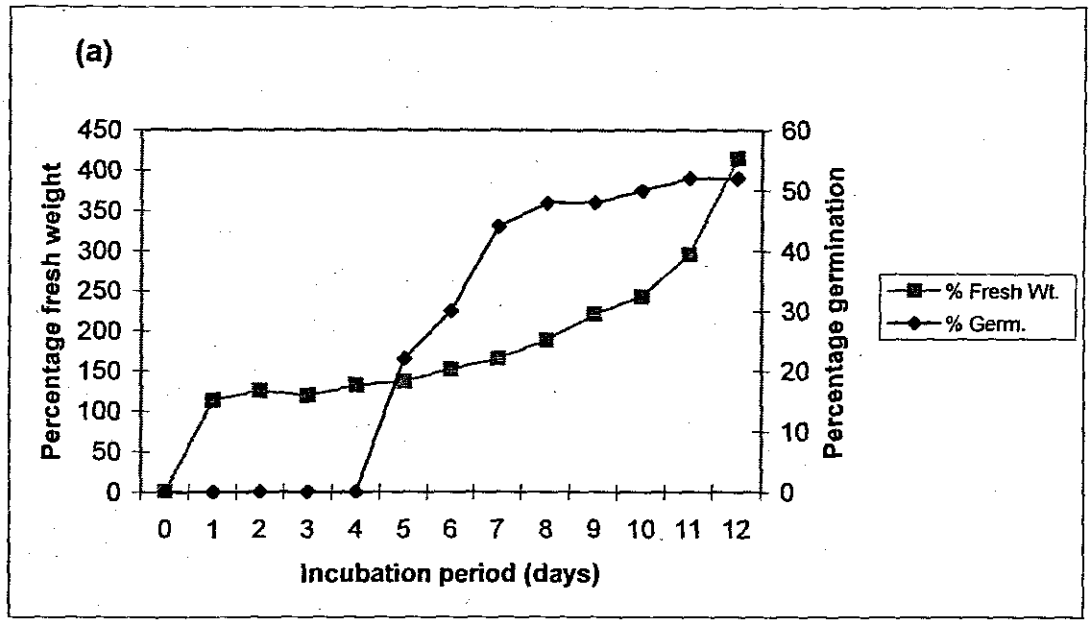
Table 4.9: Fresh and dry weight of dormant *C. palmata* and *L. sphaerica* seeds

Species	Fresh Wt. (g)	SD	Dry Wt (g)	SD	Difference
C. palmata	0.016	0	0.0153	0.001	0.0007
L. sphaerica	0.0713	0.004	0.068	0.003	0.0033

Legend: Wt: weight in grams; SD: standard deviation

b) Germinating seeds' percentage fresh weight and percentage germination of *C. palmata* and *L. sphaerica*

Table 4.10 (Appendix II) and Figure 4.11(a) illustrate that there was more than a 100% increase in seed fresh weight of *C. palmata* after one day of incubation. On day three the reduction in percentage fresh weight was not significant. In comparison with day one after incubation, there was no significant increase in fresh weight up to day five when germination (emergence of radicle) was initiating and then fresh weight gradually increased as the radicle length increased.



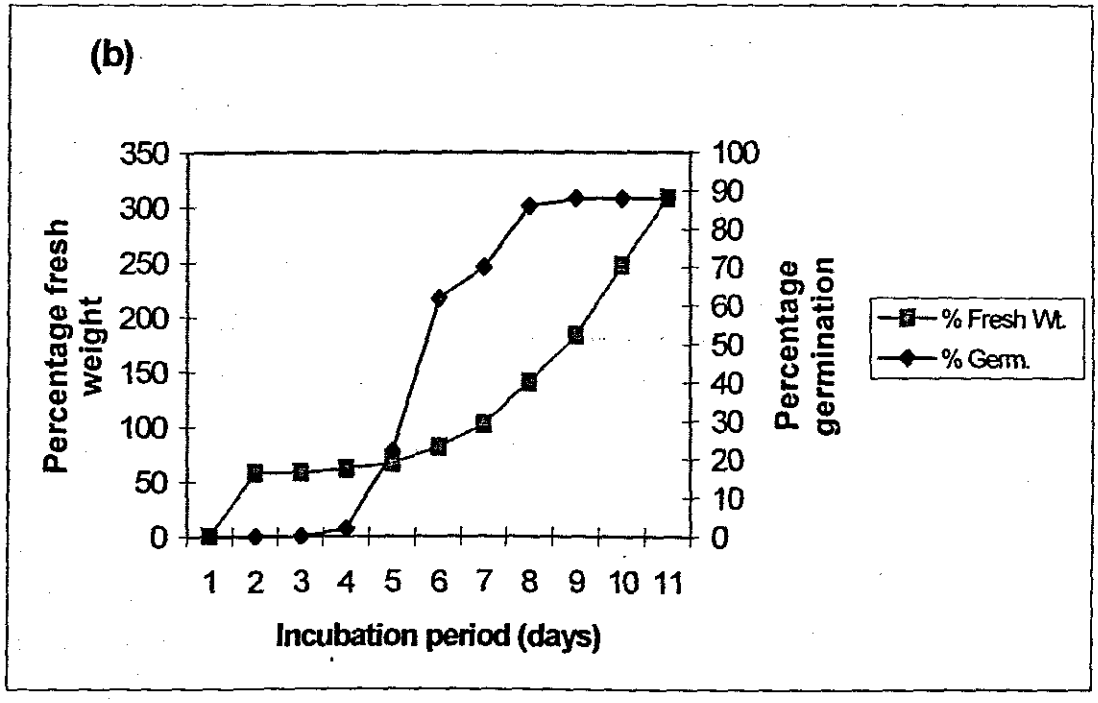


Figure 4.11: Fresh weight changes during germination of *C. palmata* (a) and *L. sphaerica* (b) seeds

Legend: % Fresh Wt.: percentage fresh weight; % Germ.: percentage germination

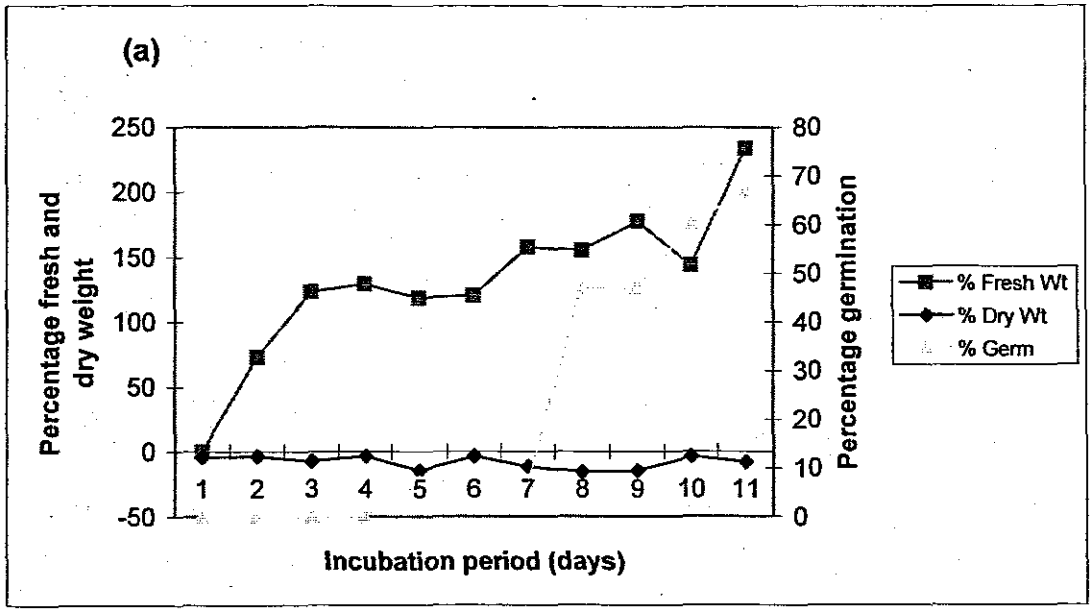
Figure 4.11a further shows that there was no significant increase in the percentage germination as from day five (noticeable germination with radicle emergence) to day 12 (termination of the experiment). The significant fresh weight increase in these two incubation periods is thus assumed to be based specifically on the seedling growth rather than germination percentage.

Table 4.10 (Appendix II) and Figure 4.11b also show that *L. sphaerica* seeds had a 58% increase in fresh weight after one day of incubation. Figure 4.11b illustrates a significant increase in percentage fresh weight on day five following initiation of germination on day four. After seven days incubation the germination percentage was 86% and thereafter very little increase in germination occurred. The sharp increase in fresh weight after six days' incubation corresponds with seedling growth.

There was a lag phase of five days before germination for both species (Figure 4.11(a & b). Determining both fresh and dry weight of the germinating seeds might indicate whether the seed's percentage fresh weight increase was due to high water content.

c) Germination, fresh and dry weight percentage of *C. palmata* and *L. sphaerica* seeds

Table 4.11 (Appendix II) and Figure 4.12a show a recorded 73% increase in fresh weight of *C. palmata* seeds after one day of incubation. As explained previously that the drying method is a destructive method, and therefore different seed batches were used for each day. There were thus many fluctuations in terms of the relationship between the tested parameters. Regardless of this fact, on day five, the day of germination, there was a decrease in percent fresh weight as well as a significant decrease in percentage dry weight.



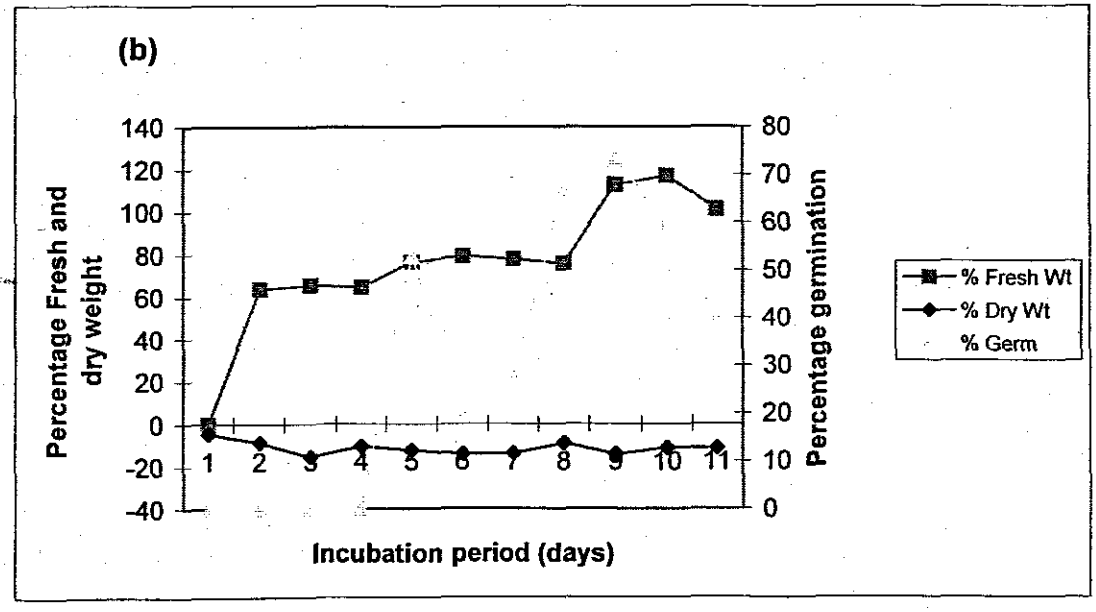


Figure 4.12: Fresh and dry weight changes during germination of *C. palmata* (a) and *L. sphaerica* (b) seeds

Legend: % Fresh Wt.: percentage fresh weight; % Dry Wt.: percentage dry weight; % Germ.: percentage germination

Table 4.11 (Appendix II) and Figure 4.12b illustrate that fresh weight after one day of incubation of *L. sphaerica* seeds increased by 63.9%. A significant decrease in percentage dry weight occurred on day two of incubation and a minor decrease in percentage fresh weight occurred on day three, before germination started on day four.

Even though there were different batches used, results obtained for *L. sphaerica* show that after germination, an increase in germination percentage caused an increase in percentage fresh weight but a decrease in percentage dry weight (Figure 4.12b, day eight).

Figures 4.12(a & b) show that even though both species germinated on the same day (day five), *L. sphaerica* had a significantly higher germination percentage (53%) than *C. palmata* (7%). These figures further show that *C. palmata* during germination had significantly higher fresh weight increase compared to *L. sphaerica* (day two to eight and 10). *C. palmata* had twice the percent fresh weight than *L. sphaerica* from day 2-10, with only a decrease on day eight and nine. There was no major difference in dry weights between these two species during germination.

4.4 Discussion

4.4.1 Seed collection, environmental and chemical factors affecting seed germination

According to the results obtained, *C. palmata* seeds collected from Eshowe and from Nkandla Forest Reserve can be stored at room temperature (about 22°C) in sealed plastic containers for months after which their viability decreases. *C. palmata* (Nkandla) show better germination at 10 months compared to eight. Their germination thus was still improving. Bewley and Black (1985) report that the storage of orthodox seeds in a cold environment (0-5°C) can retain their viability for a longer period. Cold storage might elongate the viability of *C. palmata* seeds. *C. palmata* seeds (Eshowe) also showed that younger seeds (8 month-old seeds) germinate faster, at five days, than older seeds (10 month-old seeds) at six days.

The results obtained further show that newly collected *L. sphaerica* seeds (3-4 months) germinate faster (at 3-4 days) than old (24-26 months) seeds that germinate after five to six days. The maximum percentage germination of new seeds was also high: 67% of three month-old seeds and 88% of four month-old seeds compared to 51% of 24 month-old seeds and 17.8% of 26 month-old seeds. It was noted that most of the germinated old seeds did not form healthy seedlings but had short radicles that rotted soon after their emergence.

It was more likely that the viability of *L. sphaerica* seeds increased with storage since four month old seeds had higher percent germination than three month old. The seed storage, however, must be of a limited time because their viability begins to decrease after 24 months. It seems that seed deterioration of this species is very slow as there is no significant difference in their germination percentage between three month old seeds and 24 month old seeds. The non-

significant difference between 24 month old seeds and 26 month old seeds also confirms the slow seed deterioration rate. These seeds can be stored at room temperature in sealed plastic containers for a maximum of about 24 months.

Seed storage in sealed moisture proof containers at lowered temperatures (0-5°C) can maintain the life span or viability of *C. palmata* and *L. sphaerica* seeds. Seeds were stored at room temperature (about 22°C) and uncontrolled humidity, which could have influenced viability over the longer period (Bewley and Black 1985).

Colchicine treatment lowers seed germination percentage for both species. Colchicine treatment of *Cyclamen persicum* Mill. seeds (Takamura and Mayajima 1996) and *Acacia maemsii* (de Wild) seeds (Beck *et al.* 2003) was detrimental to seed germination by increasing mortality; the effect worsened with increased concentrations and longer treatments or exposure time.

There is no difference in terms of colchicine concentration suitable for increasing germination percentage. On observation, high colchicine concentrations (0.01g/L and above) were lethal to the seeds and/or seedlings. The seeds initially germinated but had very short, abnormal radicles, producing seedlings that cannot be transplanted. According to Beck *et al.* (2003), the treatment of *Acacia maemsii* with an increased colchicine concentration and treatment exposure time influenced the reduction in root length and seedling height.

It was again observed that the exposure of the seeds to colchicine for more than one day (3DT and 10DT) affected the normal growth of seedlings in the same way as explained in the case of colchicine concentration. Ten days' treatment (10DT) also accelerated the rotting of germinated, abnormal seedlings (bleaching of cotyledons and/or first leaves). Seeds can be exposed for a limited period to high concentrations of polyploid inducer (colchicine), or vice-versa, to obtain both successful treatment and healthy plant material. According to Beck

et al. (2003), germinating seeds' (*Acacia mearnsii* (Black Wattle)) mortality rate increases with an increase in colchicine concentration as well as with an increase in exposure time.

The results obtained on germination studies of both *C. palmata* and *L. sphaerica* species showed no clear evidence on which colchicine concentrations and exposure times should be selected to continue with investigations. Using parameters such as flow cytometric analysis of roots and leaves and leaf chlorophyll contents, however, it was clear that early treatment of seeds (1DT and 3.1DT) during germination, for a maximum of one day, with intermediate colchicine concentration (0.01g/L) can be used to obtain healthy and evenly treated plants.

4.4.2 Percentage moisture content of dormant and germinating seeds

The dormant seed moisture content for both plants was very low confirming that seeds are orthodox. The moisture content of both species ranged from 4-5%. According to Bewley and Black (1985), orthodox seeds are grouped under the seeds with moisture content below four to five percent, which are immune to attack by insects and storage fungi, but may deteriorate faster than those maintained at a slightly higher moisture content.

A high increase in percentage fresh weight after one day of incubation or imbibition (water uptake by the seeds to induce germination or to break seed dormancy) is assumed to be due to the nature of orthodox seeds as they have a low moisture content. These seeds therefore have a high water or liquid uptake capacity, especially *C. palmata* that showed 100% increase in fresh weight.

According to Bewley and Black (1985) soon after the imbibition process the seeds break down their food reserves, which is evident by an increase in the rate of respiration. The breaking down of these food reserves causes a decrease in

the seeds' fresh weight. This is most likely the reason why there was a decrease in percentage fresh weight of *C. palmata* two days before radicle emergence. Food reserve degradation in *L. sphaerica* is not evident by a decrease in fresh weight, and it is therefore assumed that only the measurement of respiration rate can prove food reserve degradation in this species. The increase in percentage fresh weight after germination is assumed to be influenced by the elongation of the radicle and plumule, and hence the development of the seedling.

The results revealed that *L. sphaerica* is a fast germinating species with germination on day three of incubation as compared to *C. palmata*, germinating on day five. This, then, enables *L. sphaerica* to reach the maximum germinating percentage in a shorter period of eight days than *C. palmata* that reaches it after 11 days.

Initiation of germination (day 4) was preceded by an increase in fresh weight (after day 1). A further significant increase in fresh weight (day six) corresponds to seedling growth for both species. It is assumed that *C. palmata* needs a very high water uptake to induce germination as a result it has higher (about twice greater) percent fresh weight increase than *L. sphaerica*.

L. sphaerica had a significant decrease in percent dry weight on day two before germination started on day four (Figure 4.12b). This decrease indicates a quick breakdown of food reserves, enabling a high germination rate. *C. palmata* only shows a significant decrease in percent dry weight on day five, the day of germination (Figure 4.12a). Percent dry weight results of both species revealed that *L. sphaerica* breaks down its food reserve to induce germination quicker than *C. palmata*. This can be a reason why *L. sphaerica* germinates faster and has a high percentage germination.

It has been previously mentioned that dry weight determination is a destructive method, and therefore different seed batches were used to determine the initial,

fresh and dry weight, as well as percent germination, for each day. Using different seed batches then caused a minor fluctuation in parameters determined (percent fresh and dry weight and percent germination). This may be explained in terms of the differences in seed viability. Each seed thus has its own dormancy breaking potential. Regardless of this, *C. palmata* showed a linear increase in percent germination as compared to *L. sphaerica*.

4.5 Polyploidy induction and its effect on growth of *C. palmata* and *L. sphaerica*

4.5.1 Plant growth

This experiment was aimed to establish optimum light conditions for seedling and plant growth after polyploidy induction.

4.5.1.1 Effect of light intensity

C. palmata and *L. sphaerica* species grow in the wild under varying light intensities ranging from shade to full sun. The most suitable light intensity for plant growth was therefore determined. Plants were grown in a range of different light intensities as indicated under morphological analysis (section 3.2.6). The criteria used for determining a suitable growth condition were plant height, leaf area, stem branching (number of stems per plant), fresh and dry weight for shoots and roots.

Seedlings were produced from 11 month old *C. palmata* (Eshowe) and four month old *L. sphaerica* (KwaDlangezwa) seeds. The plants used for determining the effect of shade were 3 months old. Results appear in Table 4.12 (Appendix II) and Figure 4.13.

a) Fresh and dry weight of shoots and roots

Shoots of *C. palmata* plants grown in low light intensity had a high shoot fresh weight increase compared to those grown in full sun. A significant increase occurred at 800 µmol/m^2/s light intensity compared to >1000 µmol/m^2/s. Shoot dry weight only showed an increase at 600 µmol/m^2/s light intensity. *C. palmata* root fresh and dry weights gradually increased up to 600 µmol/m^2/s light intensity, which is also significant, and then decreased (Table 4.12 (Appendix II) and Figure 4.13a).

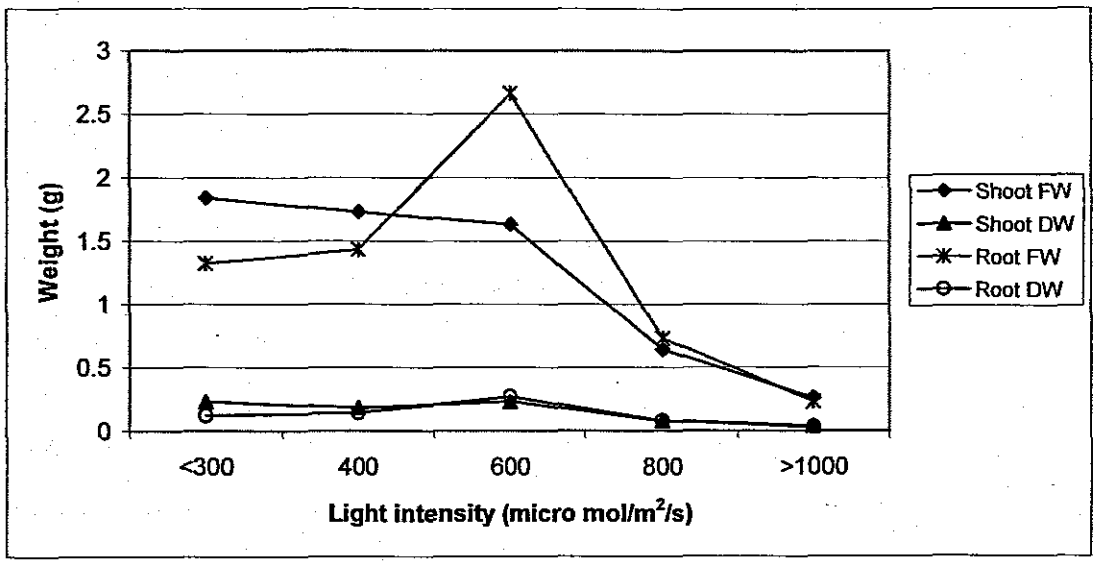


Figure 4.13a: Effect of light intensity on shoot and root fresh and dry weight of *C. palmata*

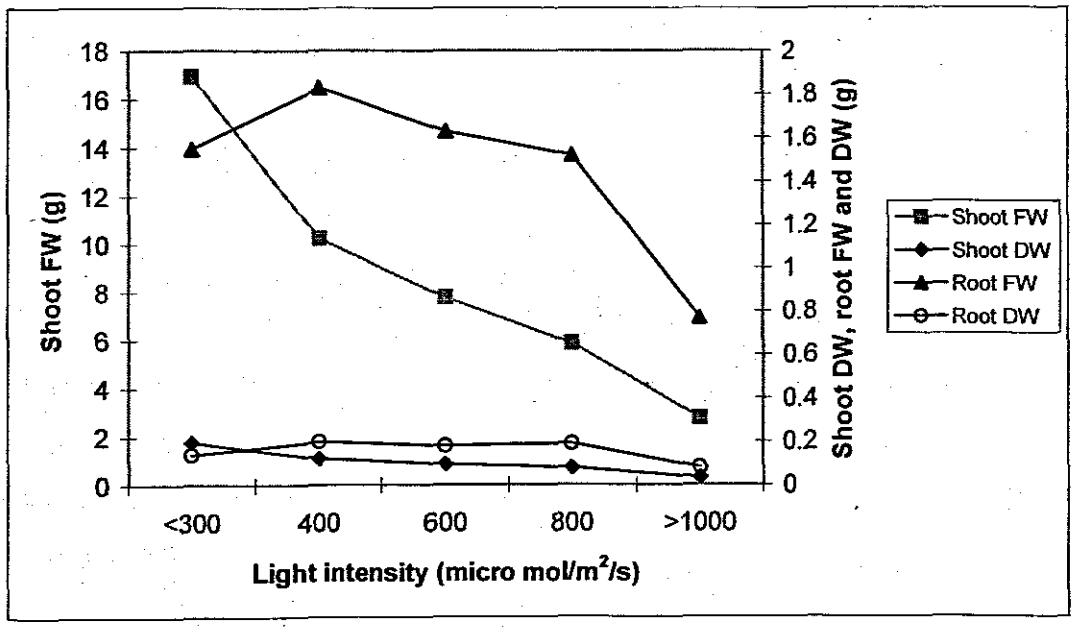


Figure 4.13b: Effect of light intensity on shoot and root fresh and dry weight of *L. sphaerica*

Legend: FW: fresh weight; DW: dry weight

Figure 4.13b showed an increase in shoot fresh weight for *L. sphaerica* plants grown in low light intensity compared with plants grown in full sun. The results showed no significant difference between plants grown in shade ranging from 400 to 800 µmol/m^2/s light intensity, but they were significantly lower than those grown in <300 µmol/m^2/s light intensity. There was only a significant increase in shoot dry weight for the plants grown at light intensity below 400 µmol/m^2/s compared with those grown above this light intensity.

There was an increase in root fresh weight for *L. sphaerica* plants grown in light intensity lower than 400 µmol/m^2/s and then a decrease as the light intensity increased. A significant increase was marked at 400 and 800 µmol/m^2/s compared to >1000 µmol/m^2/s light intensity (full sun) grown plants. Otherwise, all low light intensity grown plants did not have a significant difference in their root fresh weight. There was also no significant difference in dry weight of plants grown in full sun and low light intensity.

b) Plant height and number of stems

Results are based on three replicates per treatment. The averages of the plant height and number of stems appear in Table 4.12. Figures 4.13c, d & e reflect that there was an increase in plant height for the plants of both species that were grown in low light intensity. The increase started at plants grown at 800µmol/m^2/s. Control plants showed that *C. palmata* was shorter than *L. sphaerica* plants.

L. sphaerica plants grown at <300 µmol/m^2/s light intensity were significantly higher than those at 400 µmol/m^2/s but the other plants grown under light conditions of 400-800 µmol/m^2/s did not show any significant increase. *C. palmata* plants that were grown at 600 µmol/m^2/s light intensity were also significantly longer than those grown at 800 µmol/m^2/s.

At the age of 3 months, *C. palmata* did not show any stem branching for plants exposed to both sunlight and low light intensity. *L. sphaerica* on the other hand, showed stem branching at 400 and 600 µmol/m^2/s with significant branching starting at 400 µmol/m^2/s.

c) Leaf Area

Table 4.12 and Figures 4.13c, d & e show the effect of light intensity on leaf area. The results are based on the average of three replicates each consisting of five leaves (see section 3.2.6). Table 4.12 and Figures 4.11c, d & e illustrate an increase in leaf area of low light intensity grown plants with significance at 600µmol/m^2/s light intensity for both species. Leaves grown at <300 µmol/m^2/s further showed a significant leaf area increase compared to those grown at 600µmol/m^2/s. It was further observed from control plants that *L. sphaerica* had a bigger leaf area than *C. palmata*.

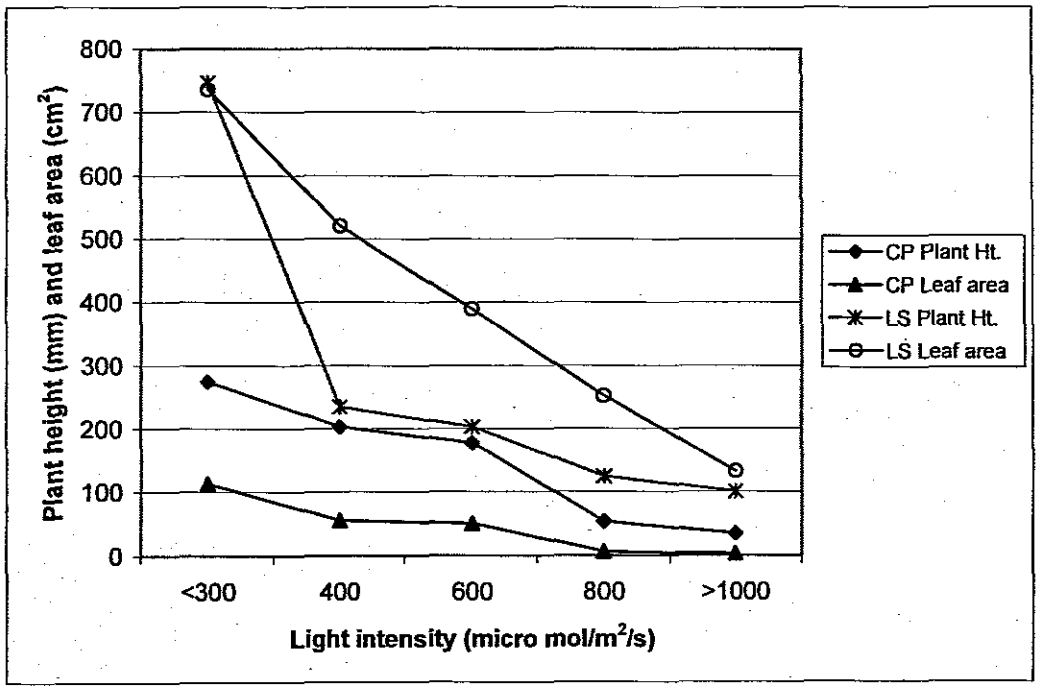


Figure 4.13c: Effect of light intensity on plant height (mm) and leaf area (cm^2) of *C. palmata* and *L. sphaerica*.

Legend: CP: *C. palmata*; LS: *L. sphaerica*

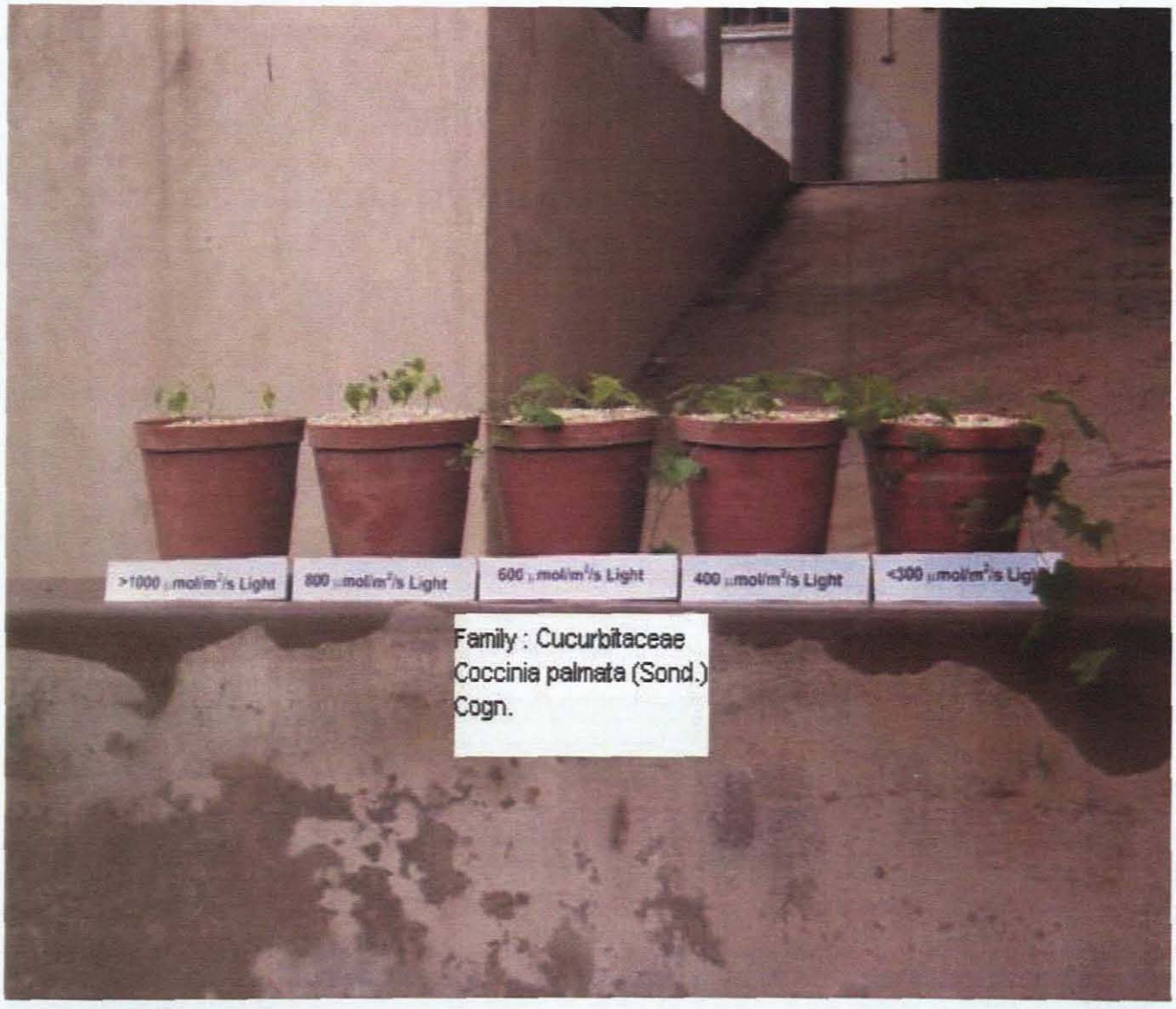


Figure 4.13d: *C. palmata* plants showing the effect of light intensity (shade) on plant height and leaf area

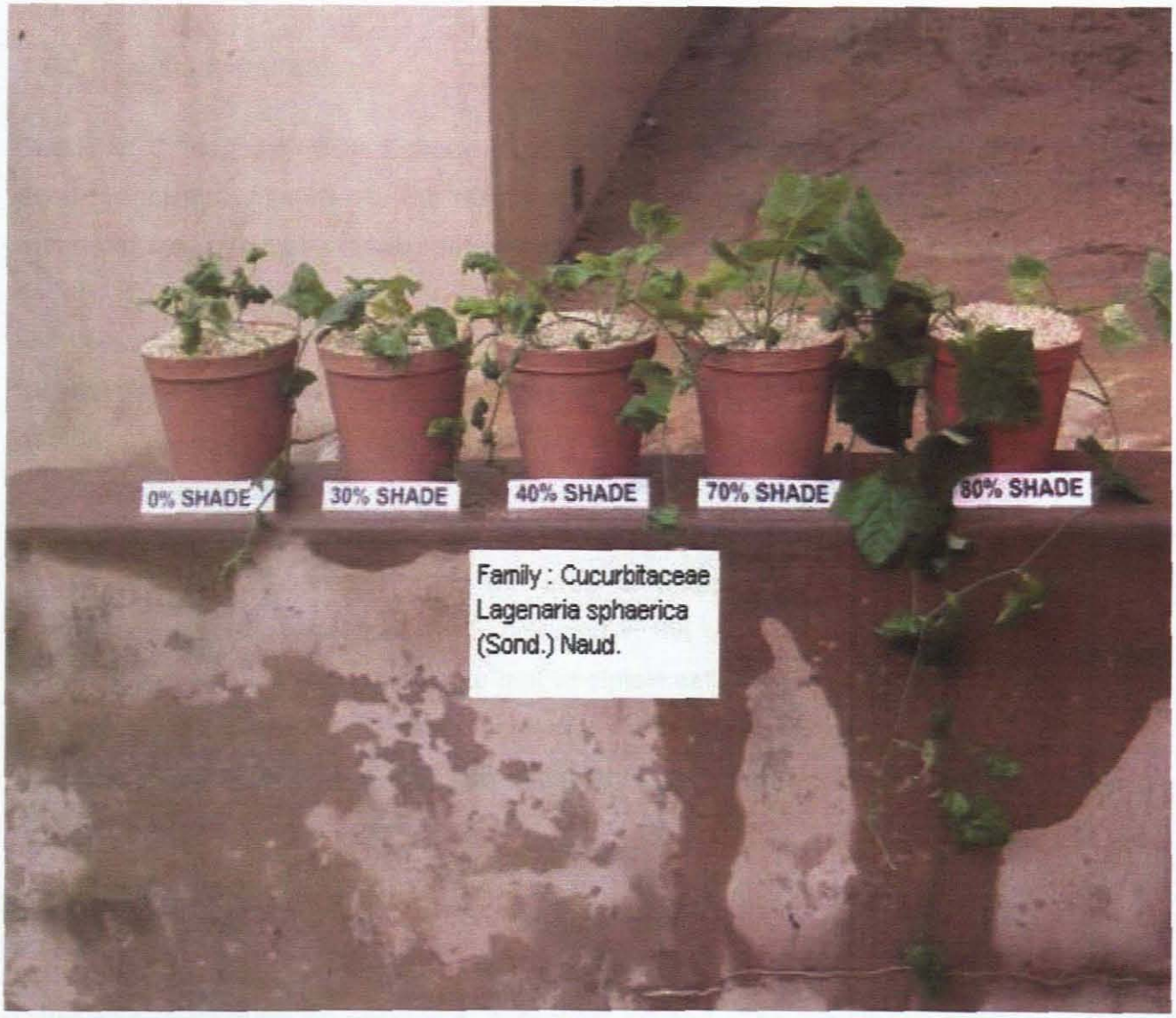


Figure 4.13e: *L. sphaerica* plants showing the effect of light intensity (shade) on plant height and leaf area

4.5.2 Polyploidy induction

Seeds of *C. palmata* (from Eshowe) and *L. sphaerica* (KwaDlangezwa) were used for polyploidy induction. The results of polyploidy induction were tested by means of flow cytometry measurements on the leaves and roots of developing seedlings (3-5 leaved seedlings). For polyploidy induction, seeds were treated with colchicine before and after incubation for germination. Five replicates (plants) were initially tested but the number was reduced depending on the number of flow cytometry histograms with consistent results for detectable ploidy level of the analysed tissue.

Ploidy ratio was determined using the area of detected cells through flow cytometry analysis. All polyploid cells detected in the presence of diploid cells had their ratio calculated in relation to that of diploid cells. However, where pure polyploids were detected, the ploidy percentage was calculated using the area. Figure 4.14 shows a sample of a flow cytometry graph showing 2n, 4n and 8n peaks.

4.5.2.1 Treatment before incubation for germination

Colchicine treatments as described in section 3.2.4(e). The results on ploidy ratios and ploidy percentages are given in Tables 4.13a & b and Figures 4.15a-f (*C. palmata*) and 4.16a-f (*L. sphaerica*). According to the results, tetraploid-diploid (4n:2n) ratios differ between roots (1.62) and leaves (0.21) in the *C. palmata* control plants. The tetraploid peak in the flow cytometry histograms, is however, the "G_2 peak" in the control since it arises from the cells with replicated chromosome but which will later divide (cells undergoing mitosis before cytokinesis).

Colchicine (1DT at 0.001 g/L) induced tetraploid-diploid chimeras (4n-2n) in *C. palmata* leaves (Figure 4.15a), but 3DT treatment (0.001 g/L) induced pure triploid (3n) and hexaploid-triploid chimeras (6n-3n) at 0.005 g/L (Table 4.16b). Concentrations of 0.01; 0.05 and 0.1 g/L induced pentaploid-diploid chimeras (5n-2n) (Figure 4.15b). The 10DT also induced 4n-2n chimeras at colchicine concentrations of 0.001 g/L, 5n-2n chimeras at 0.005 and 0.01 g/L (Figure 4.15c) and pure pentaploid (5n) at 0.1 g/L (Table 4.23b).

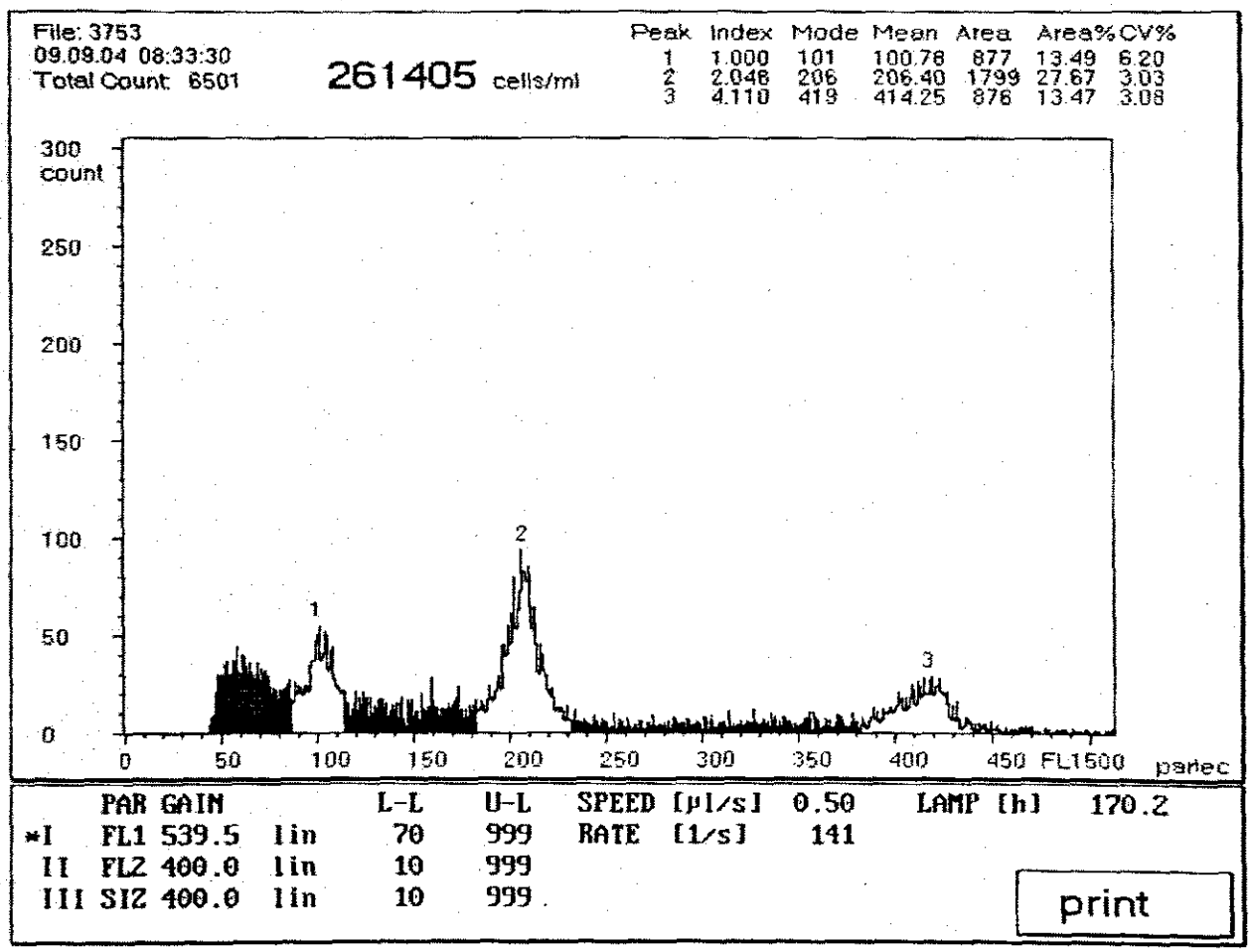


Figure 4.14: Flow cytometric analysis graph showing 2n (100), 4n (200) and 8n (400) peaks in x-axis

Table 4.13a: Ploidy ratios in relation to diploid (2n) (where 2n=1) in leaves and roots from *C. palmata* and *L. sphaerica* seeds treated with different colchicine concentrations (g/L) at different time intervals (days) before and after incubation for germination

Species	Tt.	[Colch.] g/L	Leaves					Roots		
			3n	4n	5n	6n	8n	4n	7n	8n
C. palmata	Cont.	0		0.21				1.62		
	1DT	0.001		0.5				1.56		
		0.005		0.11				1.94		1.09
		0.01						2.06		
		0.05		0.15				1.19		0.24
		0.1		0.19				2.2		
	3DT	0.001						1.31		0.81
		0.005						1.39		
		0.01			0.21			1.97		1.06
		0.05			0.21			1.61		
		0.1			0.24			1.93		
	10DT	0.001		0.45						
		0.005			0.18			1.48		0.46
		0.01			0.38			1.73		0.47
		0.05		0.21				1.58		
		0.1						1.27		
	3.1DT	0.001		0.42				1.73		
		0.01		2.17				1.91		
		0.1		1.14				1.69		0.15
	3.3DT	0.001		3.07				1.67		
		0.01		0.5				1.78		
		0.1						1.69		
	6.1DT	0.001		0.41				2.15		
		0.01		0.49				2.4		
		0.1		0.77				2.11		
	6.3DT	0.001		0.82				2.77		1.15
		0.01		0.89				2.39		
		0.1								

Legend: Tt.: treatment; Cont.: control; 2n: diploid; 3n: triploid; 4n: tetraploid; 5n: pentaploid; 6n: hexaploid; 7n: heptoploid; 8n: octoploid

Table 4.13a: Ploidy ratios in relation to diploid (2n) (where 2n=1) in leaves and roots from *C. palmata* and *L. sphaerica* seeds treated with different colchicine concentrations (g/L) at different time intervals (days) before and after incubation for germination (continued)

Species	Tt.	[Colch.] g/L	Leaves					Roots		
			3n	4n	5n	6n	8n	4n	7n	8n
C. palmata	9.1DT	0.001		1.1						
		0.01		0.29				2.1		
		0.1		0.87				1.96		
	9.3DT	0.001		0.75				2.42		
		0.01		0.21				1.35		
		0.1		1.01				2.11		
L. sphaerica	Cont.	0		0.15				1.05		
	1DT	0.001	0.07	0.28				1.34		0.56
		0.005	0.09	0.3				1.11		0.68
		0.01		0.26				1.33		0.65
		0.05	0.46	0.16				1.67		0.67
		0.1	0.12	0.07				1.27		0.64
	3DT	0.001		0.15				0.98		0.56
		0.005		1.46			0.74			
		0.01	0.22	0.11				1.34		0.29
		0.05	0.12	0.35				1.11		0.52
		0.1	0.22	1				1.45		2.47
	10DT	0.001			0.18			1.33		0.81
		0.005		0.21				2.21		1.54
		0.01		0.66			0.15	2.42	1.04	
		0.05			0.17			1.41		0.4
		0.1	2	0.32		1				
	3.1DT	0.001		1.8						
		0.01		0.42						
		0.1		0.53						

Legend: Tt.: treatment; Cont.: control; 2n: diploid; 3n: triploid; 4n: tetraploid; 5n: pentaploid; 6n: hexaploid; 7n: heptoploid; 8n: octoploid

Table 4.13a: Ploidy ratios in relation to diploid (2n) (where 2n=1) in leaves and roots from *C. palmata* and *L. sphaerica* seeds treated with different colchicine concentrations (g/L) at different time intervals (days) before and after incubation for germination (continued)

Species	Tt.	[Colch.] g/L	Leaves					Roots		
			3n	4n	5n	6n	8n	4n	7n	8n
L. sphaerica	3.3DT	0.001		0.12						
		0.01		0.33				1.48		
		0.1		4.02						
	6.1DT	0.001		0.88				2		
		0.01		0.27				2.46		
		0.1		0.77						
	6.3DT	0.001		0.48						
		0.01		0.18						
		0.1		1						
	9.1DT	0.001		0.24						
		0.01		1						
		0.1		0.39						
	9.3DT	0.001		0.95				3.6		
		0.01		0.17						
		0.1		0.21						

Legend: Tt.: treatment; Cont.: control; 2n: diploid; 3n: triploid; 4n: tetraploid; 5n: pentaploid; 6n: hexaploid; 7n: heptoploid; 8n: octoploid

Table 4.13b: Ploidy percentages of leaves and roots from *C. palmata* and *L. sphaerica* seeds treated with different colchicine concentrations (g/L) at different time intervals (days) before and after incubation for germination

Species	Tt	[Colch.] g/L	Leaves					Roots		
			2n	3n	4n	5n	6n	2n	4n	8n
C. palmata	Cont.	0	83		17			38	62	
	3DT	0.001		73			27			
		0.005		100						
	10DT	0.1				100				
	6.3DT	0.1							100	
L. sphaerica	Cont.	0	87		13			49	51	
	1DT	0.1							70	30
	3.1DT	0.001							100	
		0.01							100	
		0.1							74	26
	3.3DT	0.001							100	
	6.3DT	0.001							100	
		0.01							100	
		0.1			100				62	38
	9.1DT	0.001							85	15
		0.01			100				100	
	9.3DT	0.1								100

Legend: Tt.: treatment; Cont.: control; 2n: diploid; 3n: triploid; 4n: tetraploid; 5n: pentaploid; 6n: hexaploid; 8n: octoploid

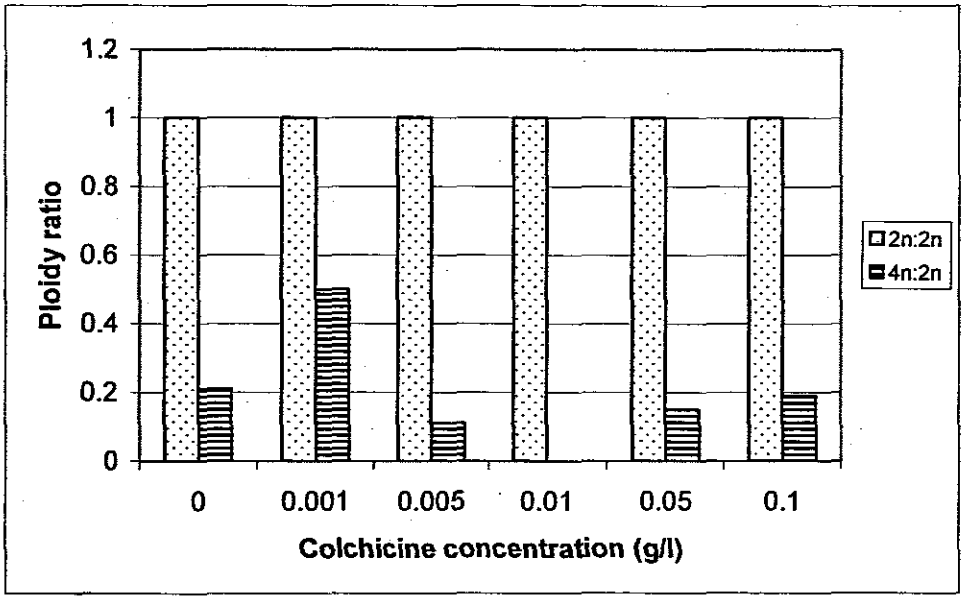


Figure 4.15a: Effect of 1DT on polyploid induction in *C. palmata* leaves

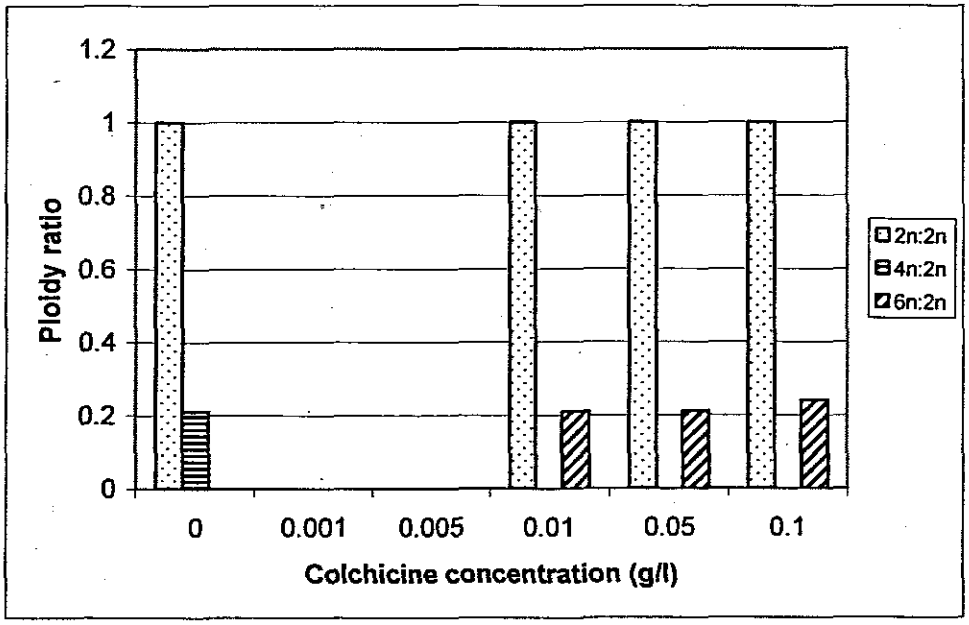


Figure 4.15b: Effect of 3DT on polyploid induction in *C. palmata* leaves

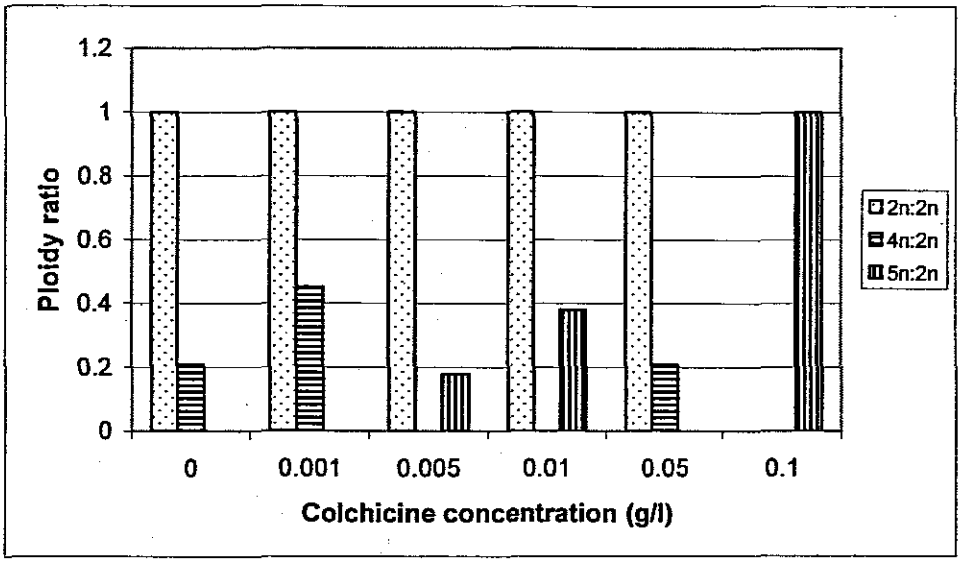


Figure 4.15c: Effect of 10DT on polyploid induction in *C. palmata* leaves

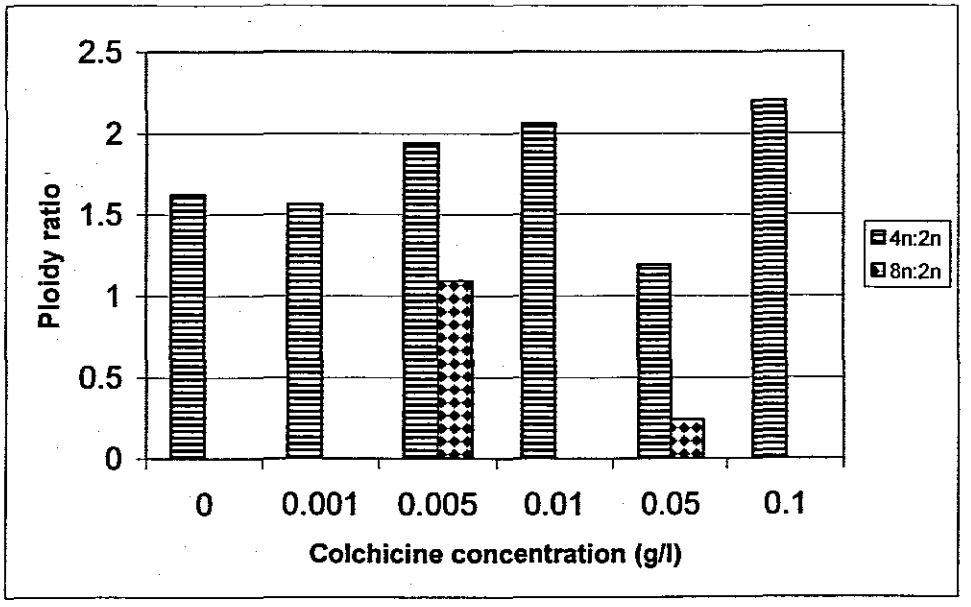


Figure 4.15d: Effect of 1DT on polyploid induction in *C. palmata* roots

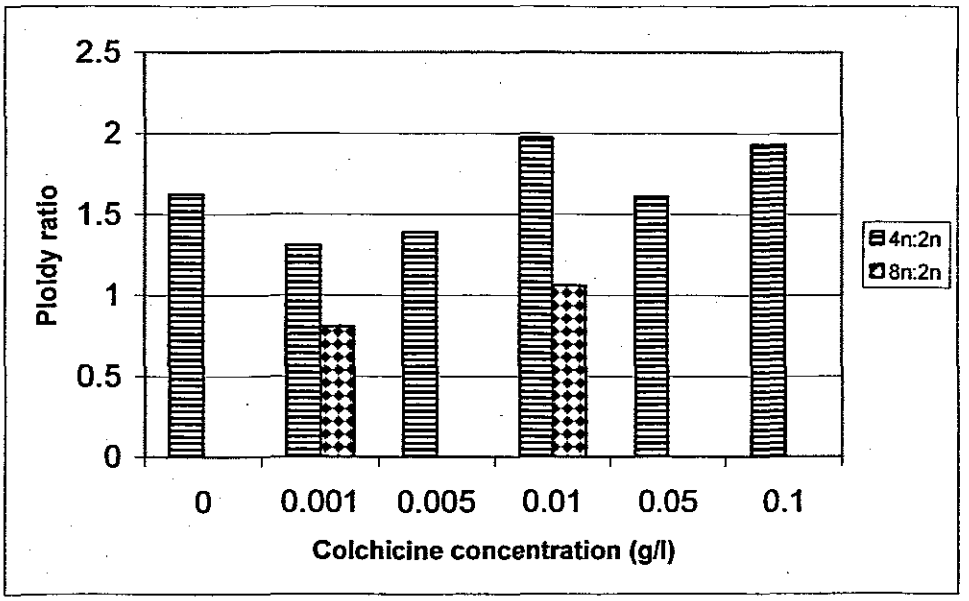


Figure 4.15e: Effect of 3DT on polyploid induction in *C. palmata* roots

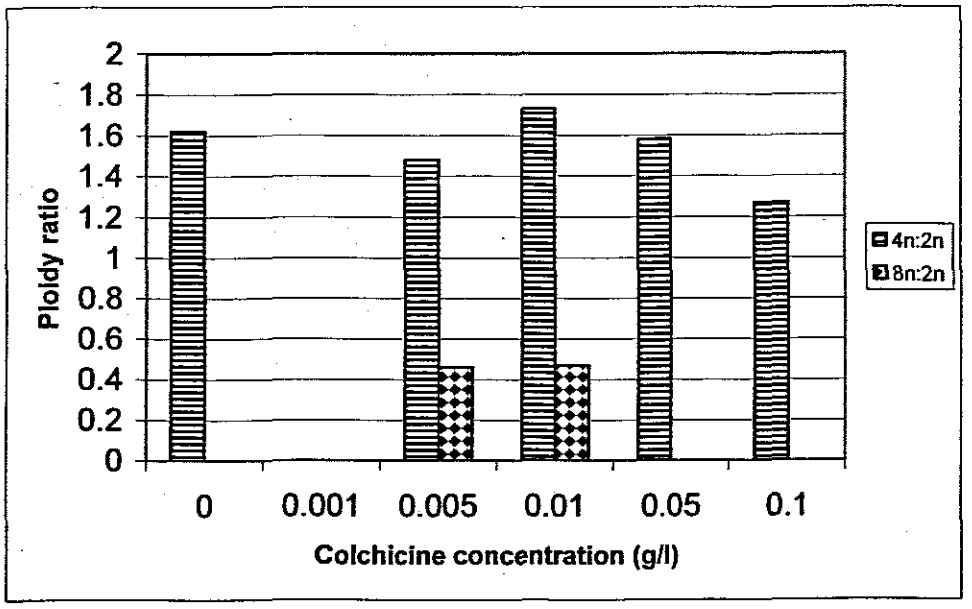


Figure 4.15f: Effect of 10DT on polyploid induction in *C. palmata* roots

C. palmata roots from 1DT with 0.001 g/L had a lower 4n:2n ratio (1.56) than the control (1.62) (Figure 4.15d). The 1DT (0.005 and 0.05 g/L) induced octoploid-tetraploid-diploid (8n-4n-2n) chimeras and 4n-2n chimeras at 0.01 and 0.1 g/L. Compared to the control (1.62), 3DT treatments with 0.01 g/L lowered the ratio to 1.39, but 0.05 g/L about equals the control (1.61) (Figure 4.15e). The 3DT treatment at 0.001 and 0.01 g/L induced 8n-4n-2n chimeras in roots. The 10DT treatment with 0.005 and 0.01 g/L also induced 8n-4n-2n chimeras while 0.05 and 0.1 g/L lowered the 4n:2n ratio to 1.58 and 1.27 respectively (Figure 4.15f).

L. sphaerica control plants also showed a higher 4n:2n ratio for roots (1.05) and lower ratio for leaves (0.5) than the control. 1DT treatment (0.01 g/L) induced 4n-2n chimeras but other concentrations induced 4n-3n-2n chimeras (Figure 4.16a). The 3DT treatment with 0.001 and 0.005g/L induced 4n-2n chimeras, with the highest ratio at 0.005 g/L (1.46), while 0.01; 0.05 and 0.1 g/L induced 4n-3n-2n chimeras (Figure 4.16b). The 10DT induced 4n-2n chimeras at 0.005 g/L, 5n-2n chimeras at 0.001 and 0.05 g/L, 8n-4n-2n chimeras at 0.01 g/L and 6n-4n-3n-2n chimeras at 0.1 g/L (Figure 4.16c).

The 1DT (Figure 4.16d) and 3DT (Figure 4.16e) at all colchicine concentrations induced 8n-4n-2n chimeras in *L. sphaerica* roots. The 10DT treatment (Figure 4.16f) induced 8n-4n-2n chimeras at 0.001; 0.005 and 0.05 g/L and 0.01 g/L induced heptoploid-tetraploid-diploid (7n-4n-2n) chimeras. Treatment with 0.1 g/L however, induced pure tetraploid (with 8n-4n chimeras) (Table 4.16b).

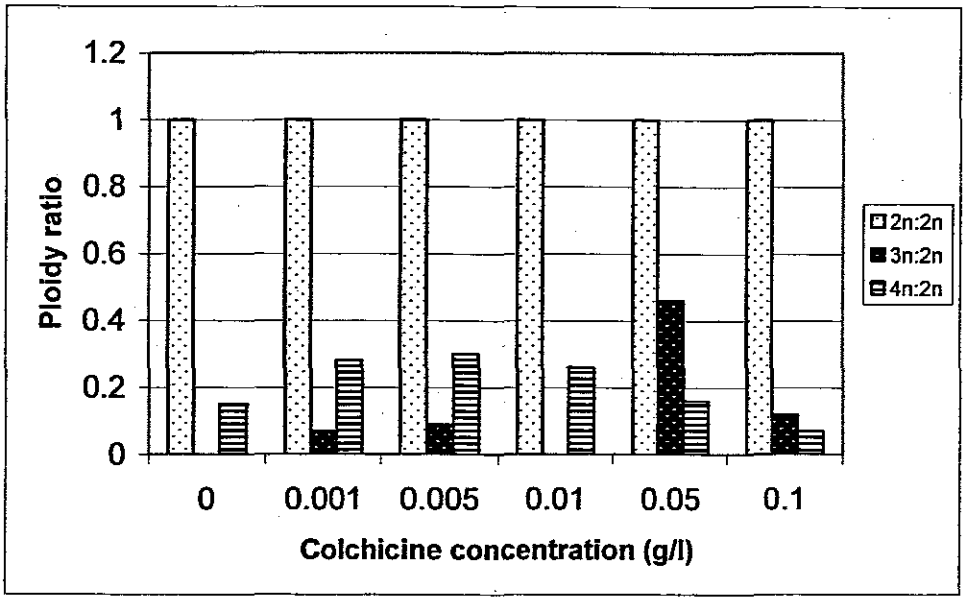


Figure 4.16a: Effect of 1DT on polyploid induction in *L. sphaerica* leaves

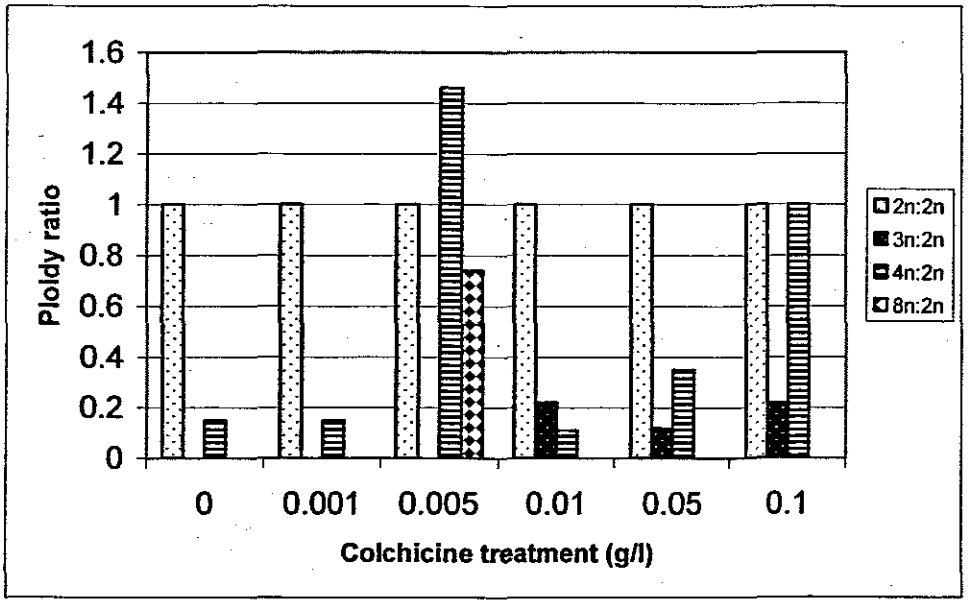


Figure 4.16b: Effect of 3DT on polyploid induction in *L. sphaerica* leaves

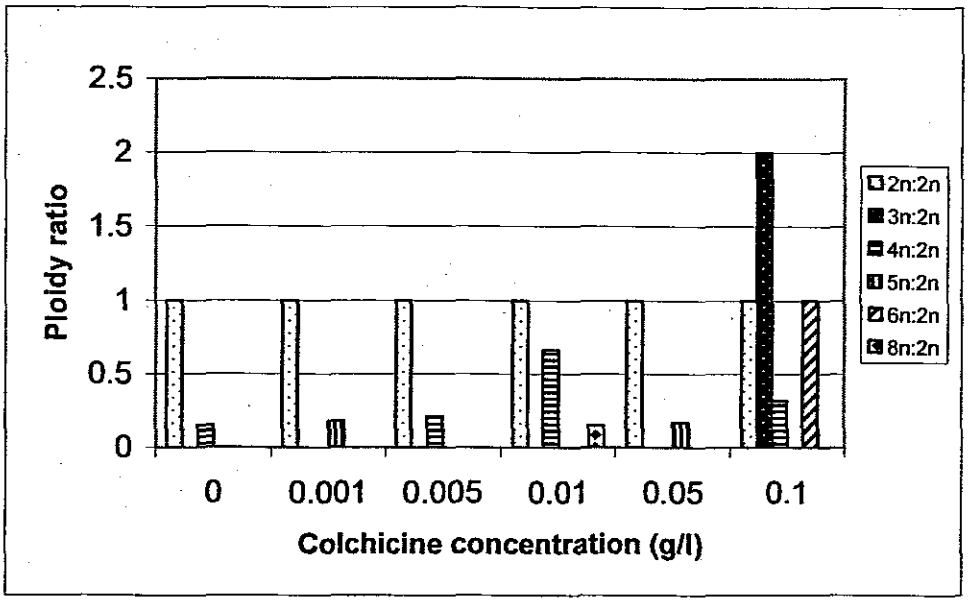


Figure 4.16c: Effect of 10DT on polyploid induction in *L. sphaerica* leaves

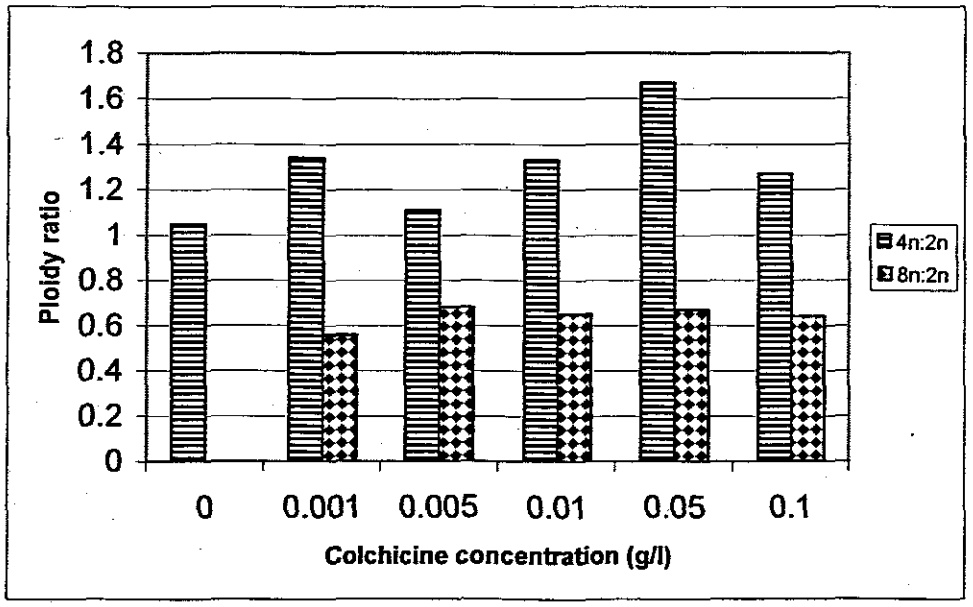


Figure 4.16d: Effect of 1DT on polyploid induction in *L. sphaerica* roots

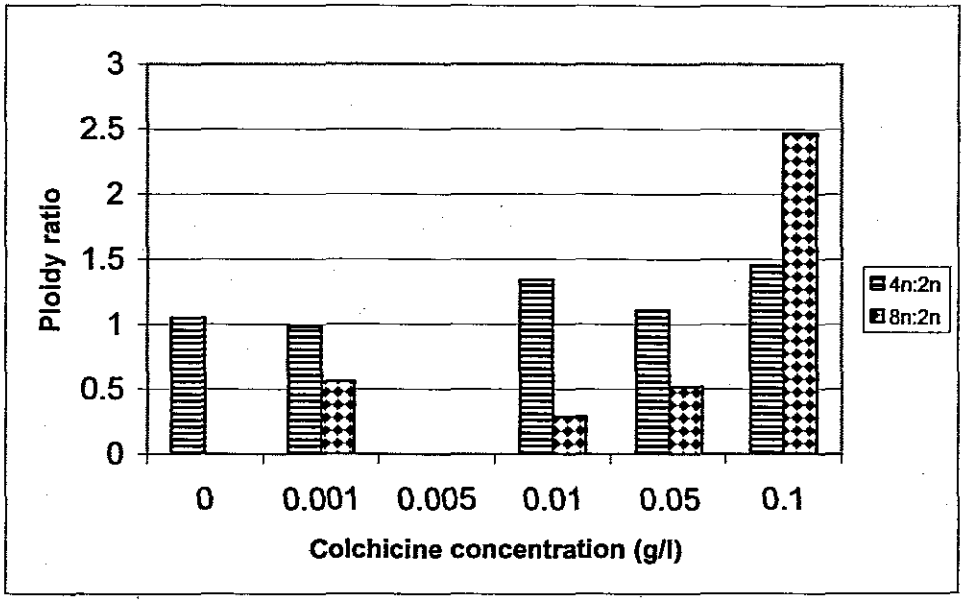


Figure 4.16e: Effect of 3DT on polyploid induction in *L. sphaerica* roots

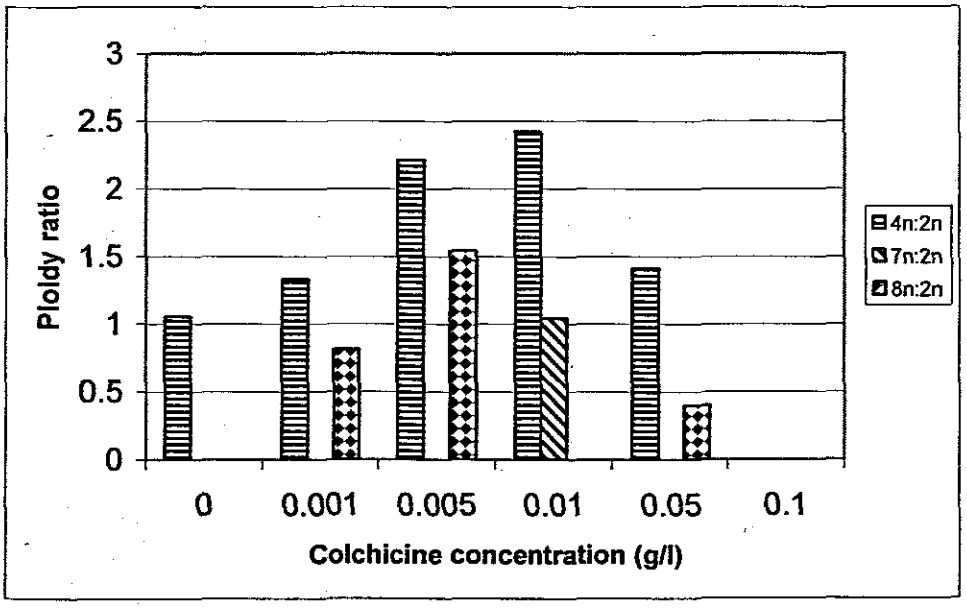


Figure 4.16f: Effect of 10DT on polyploid induction in *L. sphaerica* roots

4.5.2.2 Treatment after incubation for germination

Table 4.13a illustrates that in *C. palmata* leaves, 3.1DT induced 4n-2n chimeras at all colchicine concentrations. There was also an increase in 4n:2n ratio as the concentration increases with the highest colchicine concentration at 0.01 g/L (2.17). The roots also had induced 4n-2n chimeras at 0.001 and 0.01 g/L colchicine and 8n-4n-2n at 0.1 g/L colchicine. The 3.3DT treatment induced 4n-2n chimeras at all colchicine concentrations in both leaves and roots, with the highest ratio at 0.001 g/L for leaves and 0.01 g/L for roots.

In both leaves and roots of *C. palmata*, 6.1DT induced 4n-2n chimeras at all colchicine concentrations. The ratio (4n:2n) increased with an increase in colchicine concentration for leaves and the roots had a significantly higher ratio at all concentrations as compared to the control. Colchicine treatment also induced 4n-2n chimeras in leaves at all concentrations at 6.3DT treatment. In the roots, this treatment induced 4n-2n chimeras at 0.01 g/L, 8n-4n-2n chimeras at 0.001 g/L and pure tetraploids (4n) at 0.1 g/L (Table 4.13b). However seeds treated at this (0.1 g/L) concentration gave rise to seedlings that died soon after their transplantation (approximately about 5 days after) and before having true leaves.

In *L. sphaerica* leaves, 3.1DT induced 4n-2n chimeras with the highest 4n:2n ratio at 0.001 g/L and pure tetraploids in roots with 4n-8n chimeras with 0.1 g/L (Tables 4.13a and b). The chimera with 0.1 g/L can also be explained in terms of the highly dividing, induced tetraploid cells with its G_2 peak. In leaves, 3.3DT induced 4n-2n chimeras at 0.01 and 0.1 g/L, with the highest ratio at 0.1 g/L while in roots it induced 4n-2n chimeras at 0.01 g/L and pure tetraploids at 0.001g/L.

The 6.1DT induced 4n-2n chimeras at all colchicine concentrations in both leaves and roots, with the highest ratio at 0.001 g/L in leaves and 0.01 g/L in

roots. Again, 6.3DT treatment induced 4n-2n chimeras at 0.001 and 0.01 g/L and pure tetraploids at 0.1 g/L in leaves, while roots had pure tetraploids at all concentrations, with also a 8n-4n chimera at 0.1 g/L.

4.5.2.3: Specific day treatment and colchicine concentration treatments before and after incubation for germination

From sections 4.5.2.1 and 4.5.2.2 it was clear that further testing was necessary for the most effective colchicine concentrations and treatment duration before and after incubation. Therefore, 1DT and 3.1DT with 0.001; 0.01 and 0.1 g/L and 6.1DT with 0.01 g/L colchicine concentrations were used.

Table 4.14a indicates that in *L. sphaerica*, 1DT treatment induced 4n-2n chimeras in both leaves and roots. In leaves, the ratio of induced tetraploid cells increased with an increase in colchicine concentration. The roots had the same 4n:2n ratio (0.01 g/L) as the control or lower (0.001 and 0.1 g/L) ratio than the control. In *C. palmata*, this treatment induced 4n-2n chimeras in leaves (Table 4.14a) and pure tetraploids (100%) in roots (Table 4.14b). There was, however, a high germinating seedling mortality rate at 0.1g/L colchicine concentration.

Further, Table 4.14a shows that 3.1DT treatment with colchicine induced 4n-2n chimeras in *C. palmata* leaves and the ratio of induced tetraploid cells increases with the increase in colchicine concentration. The 6.1DT treatment with colchicine induced 4n-2n chimeras in *C. palmata* leaves (Table 4.14a) and pure tetraploids in roots (Table 4.14b). In *L. sphaerica*, 3.1DT induced 5n-2n chimeras at 0.001 g/L in leaves and in roots it did not induce chimeras or pure polyploids, but lowered the 4n:2n ratio at 0.01g/L compared with the control. The 6.1DT treatment induced 4n-2n chimeras in *L. sphaerica* leaves and did not induce any tetraploids in *L. sphaerica* roots but 6.1DT showed a lower ratio of tetraploid cells compared with the control.

Table 4.14a: Ploidy ratio in relation to 2n (where 2n=1) in leaves and roots from *C. palmata* and *L. sphaerica* seeds treated with different colchicine concentrations (g/l) one day before and after three and six days incubation for germination.

Species	Tt.	[Colch.] g/L	Leaves		Roots
			4n	5n	4n
C. palmata	Cont.	0			0.99
	1DT	0.001	0.87		
		0.01	0.85		
		0.1			
	3.1DT	0.001			
		0.01	0.26		
		0.1	1.72		
	6.1DT	0.01	0.54		
L. sphaerica	Cont.	0	0.07		0.54
	1DT	0.001			0.2
		0.01	0.11		0.54
		0.1	0.41		0.45
	3.1DT	0.001		0.08	
		0.01			0.32
		0.1			
	6.1DT	0.01	0.1		0.38

Table 4.14b: Ploidy percentages of roots from *C. palmata* seeds treated with different colchicine concentrations (g/l) one day before and after six days incubation for germination.

Tt.	[Colch.] g/L	Roots	
		2n	4n
Cont.	0	50	50
1DT	0.001		100
	0.01		
	0.1		1000
6.1DT	0.01		100

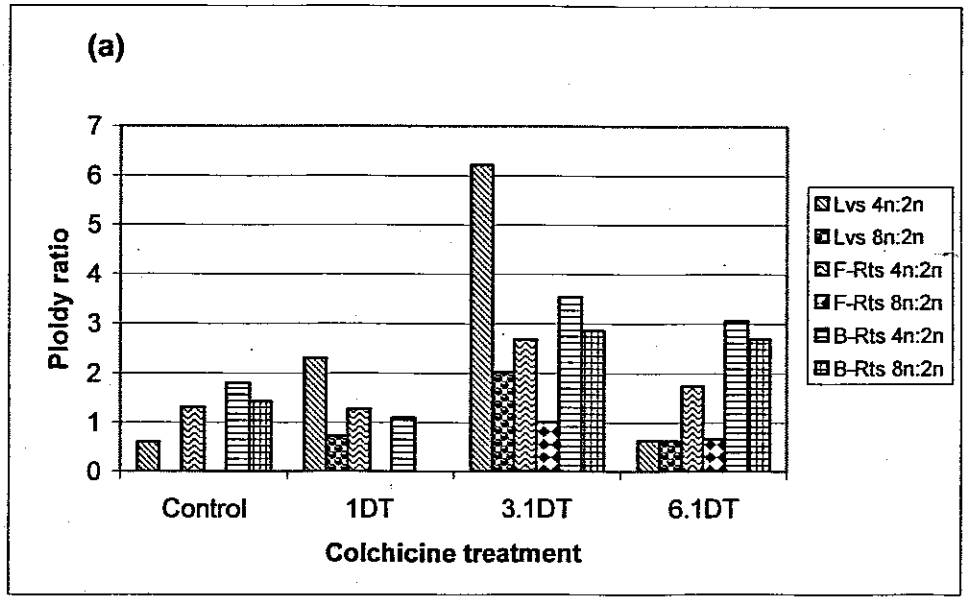
Legend: Tt.: treatment; Cont.: control; [Colch.]: colchicine concentration in g/l; 2n: diploid; 4n: tetraploid; 5n: pentaploid

4.5.2.4: One day treatment with selected colchicine concentration before and after incubation for germination

It was not clear which concentration was suitable for inducing more polyploids in both plants from the results obtained in section 4.4.2.3. It was then decided to select 0.01g/L colchicine concentration for 1DT, 3.1DT and 6.1DT to continue with the investigation since this concentration had high tetraploid induction in *C. palmata* (Figure 4.15d) and *L. sphaerica* (Figure 4.16d) roots for 1DT. This concentration also proved to have high 4n:2n ratio for leaves and roots of both species for treatment 3.1DT and 6.1DT as indicated in Table 4.13a, and had normal seedling growth.

Table 4.15 (Appendix II) and Figure 4.17a indicate that *C. palmata* leaves from all treatments (1DT, 3.1DT and 6.1DT) had 8n-4n-2n chimeras. However, the 8n cells could be the G_2 peak of the induced tetraploid cells or the tetraploid cells that have undergone spindle arrest to produce octoploids. The highest tetraploid and octoploid induction was found at 3.1DT, followed by 1DT and lastly 6.1DT.

C. palmata at this stage had both fine and bulbous roots. As a result, these roots were treated separately. The control had fine roots with normal tetraploid G_2 peak but the bulbous roots had octoploid cells. The 1DT had an induced 4n:2n ratio in both fine and bulbous roots that was lower than the control. The 3.1DT and 6.1DT had induced 8n-4n-2n chimeras while the highest polyploidy-diploid ratio was at 3.1DT in both fine and bulbous roots.



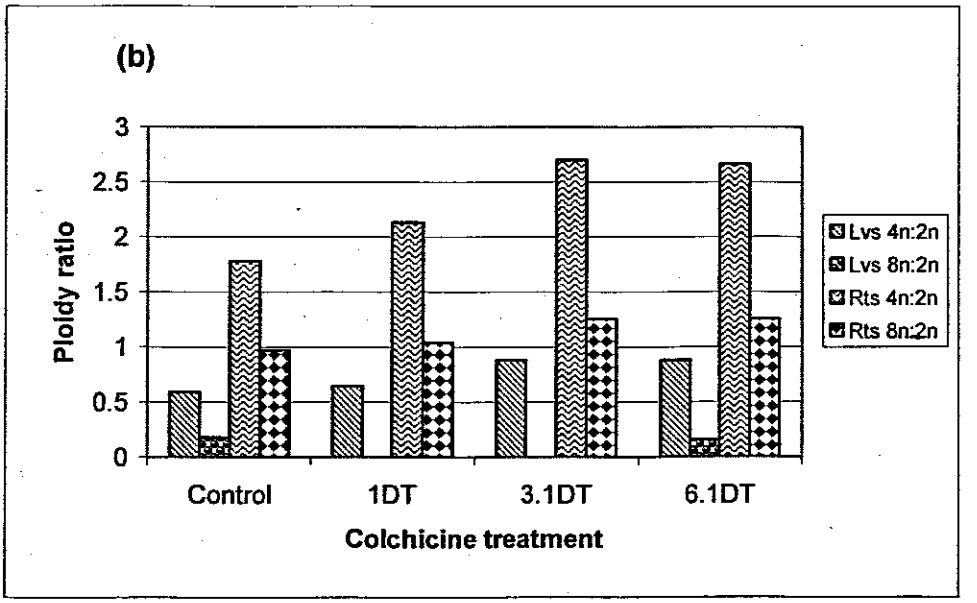


Figure 4.17: Ploidy ratio in relation to 2n (where 2n=1) in leaves and roots from one day treated *C. palmata* (a) and *L. sphaerica* (b) seeds before (1DT) and after three (3.1DT) and six (6.1DT) days incubation for germination

Legend: Lvs: leaves; Rts: roots; 4n: tetraploid; 8n: octoploid

According to the results presented in Table 4.15 (Appendix II) and Figure 4.17b, the control for *L. sphaerica* had leaves and roots with octoploid cells, which could be a normal G_2 peak of induced tetraploid cells. In leaves, 1DT and 3.1DT treatments induced 4n-2n chimeras while 6.1DT treatment induced 8n-4n-2n chimeras but the ratio of 8n:2n was even lower than that of the control. In roots, all treatments induced 8n-4n-2n chimeras where the highest polyploidy:diploid ratio was at 3.1DT.

4.5.2.5 Effect of one day treatment before (1DT) and after three (3.1DT) days' incubation on germination

It is clear from section 4.5.2.4 that 3.1DT had higher polyploidy:diploid ratio compared to 6.1DT especially for *C. palmata*. It was therefore decided that 6.1DT be excluded from the investigation. Results shown in Table 4.16 and Figure 4.18a and b indicated that colchicine treatment induced 4n-2n chimeras in leaves, with the highest 4n:2n ratio at 1DT for *C. palmata* and 3.1DT for *L. sphaerica*. In the control, roots had octoploid cells in both species. The roots had 8n-4n-2n chimeras in both treatments and the highest ratio was at 1DT for *C. palmata* and 3.1DT for *L. sphaerica*.

Table 4.16: Ploidy ratio in relation to 2n (where 2n=1) in leaves and roots from one day treated *C. palmata* and *L. sphaerica* seed before and after three days incubation for germination

Species	Tt.	Leaves	Roots	
		4n	4n	8n
C. palmata	Cont.	0.55	1.66	0.66
	1DT	1.17	2.30	1.12
	3.1DT	0.57	2.27	1.29
L. sphaerica	Cont.	0.28	2.53	0.89
	1DT	0.36	2.58	0.71
	3.1DT	0.78	3.21	1.1

Legend: Tt.: treatment; Cont.: control; 2n: diploid; 4n: tetraploid; 8n: octoploid

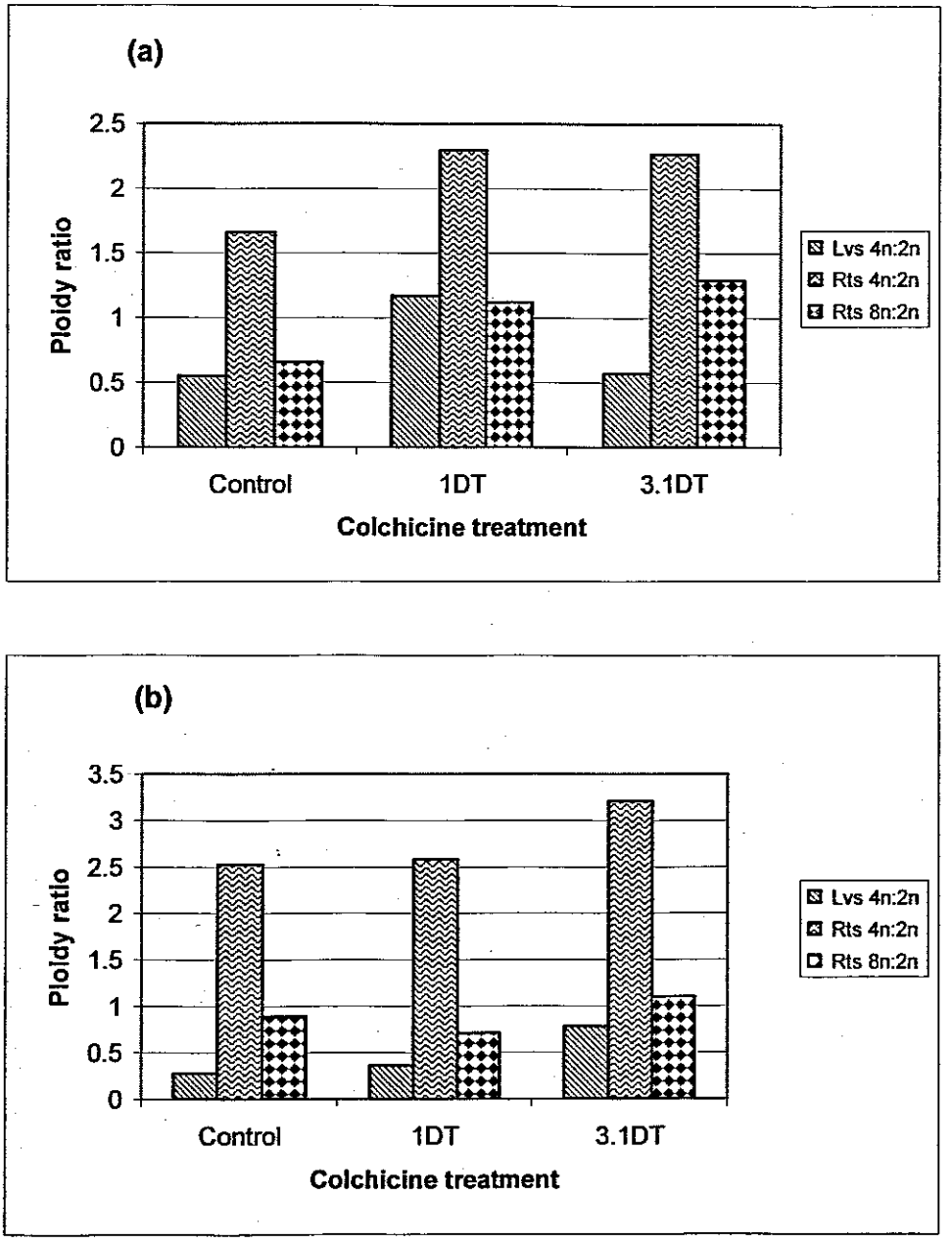


Figure 4.18: Ploidy ratio in relation to 2n (where 2n=1) in leaves and roots from one day treated *C. palmata* (a) and *L. sphaerica* (b) seeds before (1DT) and after three (3.1DT) days incubation for germination

Legend: Lvs: leaves; Rts: roots; 4n: tetraploid; 8n: octoploid

4.5.2.6 Discussion

According to the results obtained, seed treatment before incubation in both *C. palmata* and *L. sphaerica* species resulted in a high polyploid-diploid chimera ratio for the roots compared to the leaves. Hypocotyl cells divide earlier than epicotyl cells (Bradbeer 1988, Bewley and Black 1985, Raven *et al.* 1992, 1999) and this may explain this result.

The 1DT treatment either killed the hypocotyl cells or had no effect on *C. palmata* leaves, since it induced 4n-2n chimeras at 0.001g/L only, but induced tetraploid and octoploid cells in the roots. In *L. sphaerica,* 1DT induced chimeras with triploids for all concentrations except 0.01g/L in leaves, which are sterile polyploids, but induced 8n-4n-2n chimeras in roots. This treatment is initiated at the stage of seed imbibition and is terminated when only the hypocotyl cells are dividing. The induction of 3n may arise from the dividing 2n cells under the influence of colchicine, that will eventually produce 3n and haploid (n) cells.

An increase in colchicine concentration at 1DT treatment caused an increase in polyploid cell induction specifically for the roots, especially in *C. palmata.* It also increased the mortality rate of the seedlings soon after radicle protrusion and when they were transplanted before forming true leaves. Tilney-Bassett (1986), Takamura and Mayajima (1996) and Beck *et al.* (2003) confirm that colchicine treatment is detrimental to seed germination by increasing mortality; the effect worsened with increased concentrations and longer treatments (exposure time). Tilney-Bassett (1986) further reports that colchicine is toxic and overdosing kills the tissues.

Longer colchicine treatments (3DT and 10DT) induced sterile polyploids, for example, triploids, pentaploids and heptoploids, or either increased or decreased the ratio of tetraploid- and/or octoploid-diploid chimeras, especially in leaves of both species. The induction of triploids and pentaploids may arise from the

dividing induced tetraploids, which after chromosome replication during mitotic division, will divide and give triploids and pentaploids.

It is clear from the results that longer exposure (more than a day) to colchicine may cause the induction of octoploid cells from initially induced tetraploid cells. Tilney-Bassett (1986) confirms that if meristematic cells have prolonged contact with colchicine, the chromosome may pass through a further cycle of DNA synthesis and replication before once again becoming arrested at the next metaphase. So, in the first round of chromosome replication, diploid cells become tetraploid, after spindle arrest, and in the second round the tetraploid cells, in turn, become octoploid. The lowering in 4n:2n or 8n:2n ratio may be caused by cells being killed by prolonged colchicine treatments (Tilney-Bassett 1986).

Seed treatment after incubation eliminated the induction of 3n, 5n and 7n polyploids in both species. Longer treatments (3 days) after incubation either induced high polyploid-diploid ratio or pure polyploids (tetraploids and octoploids), especially in roots, when induced at an early stage of germination (3.3DT), but most of long-treated seedlings died soon before their transplantation. However, longer treatments at the later stages of germination (6.3DT and 9.3DT) killed some of the germinating seedlings by softening or bleaching the cotyledons or true leaves.

An increase in colchicine concentration within a treatment either increased or decreased the ratio of polyploid-diploid cells. The increase in ratio with increase in concentration was mostly found when the incubated/germinating seeds were treated for one day. This suggests that an efficient treatment is attained when shorter exposure time is accompanied with high colchicine concentrations. Again, longer exposure time in high colchicine concentrations killed the cells (Tilney-Bassett 1986, Takamura and Mayajima 1996, Beck *et al.* 2003) with evidence of decreased polyploid-diploid ratio. Seed treatment early in the

incubation (3 days incubation) can, however, be accompanied with high colchicine concentrations to induce more polyploids. The problem remains with a high mortality rate that was normally found in high concentrations (for example 0.1g/L).

It is clear from the results that the maximum exposure time to colchicine treatment is one day, either before or after incubation. After further selection, as represented in the results, it was clear that even though 0.1g/L colchicine induced chimeras with more polyploid cells (and even pure polyploids) it caused a high mortality rate either in cells or germinating seedlings. Treatment with colchicine at 0.001g/L, however, did not have any significant effect compared to the control in terms of polyploid-diploid ratio. As a result, 0.01g/L colchicine seemed to be the optimum concentration to be used. Since most of 6.1DT treated seedlings were bleached or killed and some flow cytometric, morphology and physiology results were not significantly different from those of the control, 6.1DT was then excluded.

The induction of octoploid cells in both *C. palmata* and *L. sphaerica* control roots and leaves indicates that polyploidy occured due to natural or environmental factors such as heat stress or temperature changes (Broertjies and van Harten 1988, Ramsey and Schemske 1998). As these plants were grown in pots under nursery conditions, the heat that was generated by the pot to the plant roots could have had a drastic effect in changing the ploidy status of the roots. As the nursery is not well aerated because it was surrounded by shade cloth, variation in temperature under this condition could change the ploidy status of the leaves.

The 1DT and 3.1DT with 0.01g/L colchicine treatment seemed to be the best to induce polyploid cells in roots and leaves of both plants. According to the results, however, 1DT treatment induces more polyploid cells in *C. palmata* roots and leaves while 3.1DT treatment induces more in *L. sphaerica*, though there are some minor exceptions for both cases.

4.5.3 Effect of polyploidy induction on growth of *C. palmata* and *L. sphaerica*

The effect of 1DT and 3.1DT on polyploidy induction was investigated on leaves and flowers of both *C. palmata* and *L. sphaerica* species.

4.5.3.1 Effect of 1DT and 3.1DT on polyploid tissue formation in leaves and flowers of *C. palmata* and *L. sphaerica*

The plants used for this analysis were 11 months old. Sepals (calyx) of the flowers were used for flow cytometry analysis. Flower analysis was only done for *L. sphaerica* since it was the only species with flowers at the time of analyis. Both young and mature leaves were used for the analysis.

Table 4.17 and Figure 4.19 indicate that only 3.1DT induced 4n-2n chimeras in young and mature leaves of *L. sphaerica*, but 1DT had a 4n:2n ratio lower than that of the control. The calyx of the flowers showed the induction of 4n-2n chimeras by both 1DT and 3.1DT and the highest ratio is at 3.1DT.

According to the results presented in Table 4.17 and Figure 4.20a, young leaves of *C. palmata* did not show a significant increase in 4n:2n ratio compared to the control. The induction of 4n-2n chimeras is thus doubtful in this analysis. Mature leaves showed the induction of chimeras with the presence of 3n cells in both treatments (Figure 4.20b). 1DT induced 6n-4n-3n-2n chimeras while 3.1DT induced 8n-6n-4n-3n-2n chimeras.

Table 4.17: Ploidy ratio in relation to 2n (where 2n=1) in leaves and flowers from one day treated *C. palmata* and *L. sphaerica* seeds before and after three days incubation for germination

Species	Tt.	Young leaves	Mature leaves				Flowers
		4n	3n	4n	6n	8n	4n
C. palmata	Cont.	0.32		0.49			
	1DT	0.34	0.42	0.78	0.16		
	3.1DT	0.34	0.52	1.94	0.67	1.21	
L. sphaerica	Cont.	0.36		0.46			0.18
	1DT	0.24		0.41			0.55
	3.1DT	0.43		0.5			0.64

Legend: Tt.: treatment; Cont.: control; 2n: diploid; 3n: triploid; 4n: tetraploid; 6n: hexaploid; 8n: octoploid

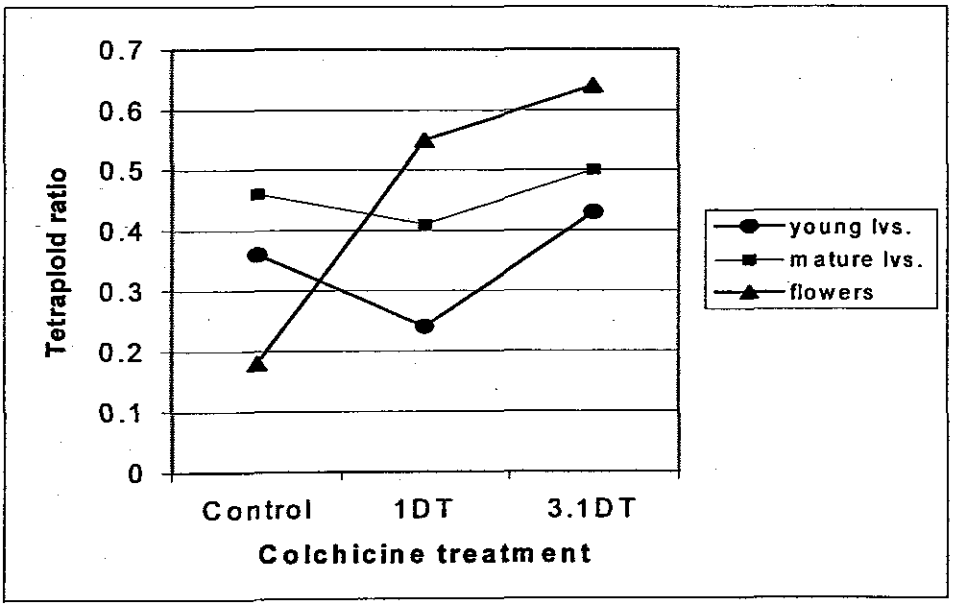


Figure 4.19: Tetraploid (4n:2n) ratio of young and mature leaves and flowers from one day treated *L. sphaerica* seeds before (1DT) and after three (3.1DT) days incubation for germination

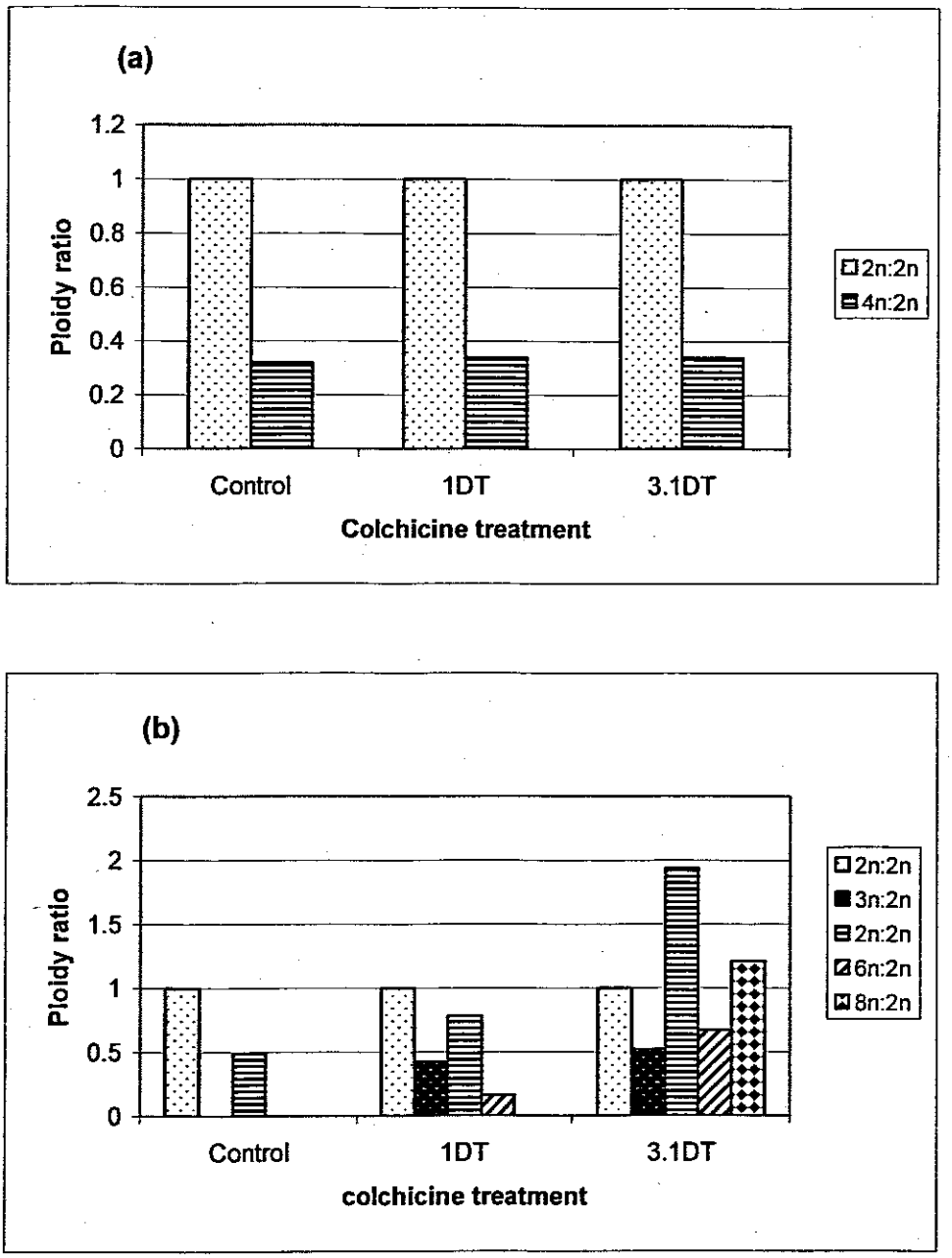


Figure 4.20: Ploidy ratio of young (a) and mature (b) leaves from one day treated *C. palmata* seeds before (1DT) and after three (3.1DT) days incubation for germination

Legend: 2n: diploid; 3n: triploid; 4n: tetraploid; 6n: hexaploid; 8n: octoploid

4.5.3.1.1 Discussion

(a) Fresh and Dry Weight

There was a linear decrease in shoot fresh and dry weight of *C. palmata* and *L. sphaerica* plants as the light intensity increased. From low light intensity, plants became etiolated. Therefore these taller or etiolated plants had increased shoot fresh and dry weight, compared to shorter plants grown at high light intensities. It is clear from the results that *L. sphaerica* had a higher shoot fresh and dry weight than *C. palmata* in both low and high light intensity conditions. This also links to the fact that *L. sphaerica* has longer stems than *C. palmata*, which then influence shoot weight.

For *L. sphaerica*, the results show that there was an increase in root fresh and dry weight up to 400 $\mu mol/m^2/s$ light intensity, and follwed by a gradual decrease with increase in light intensity. For *C. palmata,* the results show that there was an increase in root fresh and dry weight up to 600 $\mu mol/m^2/s$ light intensity, and then a gradual decrease with increase in light intensity.

Hale and Orcutt (1987) explain that plants occupying sunny habitats (sun plants) require high light intensities to maintain a high photosynthetic rate and consequently they have lower rates of photosynthesis at low light intensities. Therefore, in light intensities that are below the compensation point (such as deep shade), injury occurs in plants as a result of starvation. The most rapid effect is a decrease in carbohydrate content followed by other alterations of metabolism. This may explain why root growth of *C. palmata* and *L. sphaerica* deteriorated at light intensities lower and higher than 600 and 400 $\mu mol/m^2/s$, respectively.

(b) Plant Height and Stem Branching

It is clear from the results that *C. palmata* plants are generally shorter than *L. sphaerica* plants. Low light intensity promoted stem elongation in both *C. palmata* and *L. sphaerica* plants. On observation, plants (especially stems) exposed to very low light intensities (400 - <300µmol/m^2/s) were more brittle than those exposed to higher light intensities to full sun. This then confirmed the process of etiolation (stem elongation at reduced light intensity) that has taken place on these plants. Both species did not show any significant difference in their stem branching at different light intensities.

(c) Leaf Area

It is clear from the results that *L. sphaerica* leaves are broader than *C. palmata* ones. An increase in shade caused an increase in leaf area for both species. The increase in leaf area may be either advantageous in terms of an enlarged site for photosynthesis or disadvantageous as it is coupled with shade stress. In general, shade stress causes the plants to increase leaf area in order to overcome the low light intensity and thus be able to survive (Hale and Orcutt, 1987).

(d) Flow cytometry

According to the results obtained, treated young leaves of *C. palmata* show a very small difference in ratio of tetraploid cell induced compared to the control. However, mature leaves have a very high tetraploid ratio and further show mixed chimeras with triploids, hexaploids and octoploids. Other factors, such as temperature change, water stress and nutrient stress (Broertjies and van Harten 1988, Ramsey and Schemske 1998) might have induced polyploids in this species as indicated in mature leaves. Analysed leaves were from the plants that

were irrigated for a short period after their transplantation to the soil until they were established in the new environment. Since Cucurbitaceae are sub-tropical to tropical plants (Pooley 1998, Andres 2004), they might have experienced water stress and thus had polyploid tissue induction.

C. palmata and *L. sphaerica* plants were also fertilized with 2.3.2(22) fertilizer at least once every two months. This may not have satisfied the plant's nutrient requirements since there was no soil analysis for the area used for plantation. Nutrient stress might have induced polyploids. Temperature fluctuations arising from weather changes may also induce polyploidy.

In *L. sphaerica*, even though the 4n:2n ratio is higher in 3.1DT than for the control for both young and mature leaves, the increase was not significant. Again, the lowered ratio at 1DT was also not significant. It is therefore assumed that colchicine treatment either had a very low effect on the epicotyl or killed some dividing cells of the germinating seeds. 1DT and 3.1DT almost equally influenced tetraploid cell induction in *L. sphaerica* flowers. Nevertheless, 3.1DT treatment had more induction of tetraploids than 1DT treatment.

4.5.3.2 Effect of polyploidy induction on aspects of plant morphology

The results for the effect of polyploidy induction on plant morphology were based on fresh and dry weight of shoots and roots, plant height, stem branching (number of stems per plant), foliation (number of leaves per plant) and leaf area, stomatal count and guard cell length measurement.

a) Effect of colchicine concentration and polyploidy induction on *C. palmata* and *L. sphaerica* plant morphology

Seedlings used originated from 1DT and 3.1DT at different (0.001; 0.01 and 0.1g/L) colchicine concentrations and 6.1DT at 0.01g/L colchicine concentration. Three month-old plants grown in summer (October – January) were used for this investigation. The results for plant height in millimetres, stem branching (number of stems per plant) and foliation (number of leaves per plants) appear in Table 4.18.

i) Plant height, stem branching and number of leaves

Table 4.18 shows a decrease in average plant height of all treated *C. palmata* plants for all colchicine concentrations, except 3.1DT at 0.1g/L, but the decrease was not significant. *L. sphaerica*, however, showed fluctuation in plant height, with significant decrease at 1DT (0.001g/L) and 3.1DT (0.1g/L) and the decrease was not significant at 1DT (0.1g/L). Other treatments did not show significant increase in plant height compared to the control.

Treated plants of both *C. palmata* and *L. sphaerica* did not show significant difference in stems branching and number of leaves compared to untreated plants (Table 4.18).

Table 4.18: Effect of polyploidy induction and colchicine concentration on *C. palmata* and *L. sphaerica* plant morphology

Criteria	Tt.	[Colch.] g/L	*C. palmata*		*L. sphaerica*	
			Average	SD	Average	SD
Plant height (mm)	Cont.	0	226.64	137	122.12	56
	1DT	0.001	208.63	140	29.42	17
		0.01	222.68	111	157.5	187
		0.1	219.65	109	76.67	24
	3.1DT	0.001	128.73	116	224.51	309
		0.01	204.83	214	390.31	264
		0.1	278.29	158	45	7
	6.1DT	0.01	216.75	11	131.36	107
Stem branching (stems per plant)	Cont.	0	1	0.71	1	0.38
	1DT	0.001	1	0	1	0
		0.01	2	0.53	1	0
		0.1	1	0.53	2	0.71
	3.1DT	0.001	1	0.45	1	0.82
		0.01	1	0	1	0.52
		0.1	2	0.96	1	0
	6.1DT	0.01	1	0	1	0.46
Leaves per plant	Cont.	0	12	6	8	2
	1DT	0.001	13	5	5	1
		0.01	17	6	7	4
		0.1	17	5	8	4
	3.1DT	0.001	10	6	11	9
		0.01	13	12	14	6
		0.1	20	10	6	1
	6.1DT	0.01	12	0	11	6

Legend: Tt.: treatment; [Colch.]: colchicine concentration in g/l; SD: standard deviation

b) Effect of polyploidy induction with 0.01g/l colchicine on *C. palmata* and *L. sphaerica* plant morphology

Plants from one day seed treatment before one day (1DT) and after three days (3.1DT) and six (6.1DT) days of incubation with 0.01g/l were selected to continue with investigation. These selections were based on the flow cytometry results.

Plants were grown at different seasons and they were analysed for their morphological differences as they grew. Some plants were grown from the beginning of winter to summer (April – February) (these plants are indicated by: (BW), some from mid-winter to summer (June – February) (MW) and the others from summer to the following summer (January – January) (S). Only plants grown in the beginning of winter had 6.1DT.

The results appear in Table 4.19 (Appendix II) and Figure 4.21 – 4.26

i) Effect of polyploidy on plant height of *C. palmata* and *L. sphaerica*

Plant height in millimetres was determined at the earlier stages of growth since both species are climbers and their measurement is complex at later stages. As explained in the methods, when there is more than one stem per plant, an average plant height of that particular plant was determined and then later the total average of plants per treatment was recorded.

Table 4.19 and Figures 4.21a and d indicate that all treated *C. palmata* plants grown from the beginning of winter had a decrease in plant height at an age of three months, but the decrease was not significant. At the age of four months there was also a decrease and this was significant at 3.1DT. The control, 1DT and 6.1DT each had a significant increase in plant height after four months compared to three months.

Plants grown in mid-winter also showed a decrease in plant height in both two and four months stage but this was not significant. There was a significant increase in plant height as both control and treated plants grew from two to four months but treated plants still had shorter stems compared with the control plants at the age of four months.

According to the results in Table 4.19 (Appendix II) and Figures 4.21b and c all three month-old treated *L. sphaerica* plants grown at the beginning of winter showed a decrease in plant height that was only significant at 1DT. When the plants were four months old the decrease was significant at 1DT and 3.1DT. As the plants grew from three to four months there was a significant increase in plant height for the control, 1DT and 6.1DT. 3.1DT, however, did not show a significant increase. This trend for plant growth from three to four months was clearer in *C. palmata*.

Mid-winter grown, two month old plants had increased plant height for 1DT and decreased plant height for 3.1DT but both these changes were not significant. After four months all treated plants showed no significant increase in their height. *L. sphaerica* showed a highly significant plant height increase for treated plants versus control plants as they aged from two to four months.

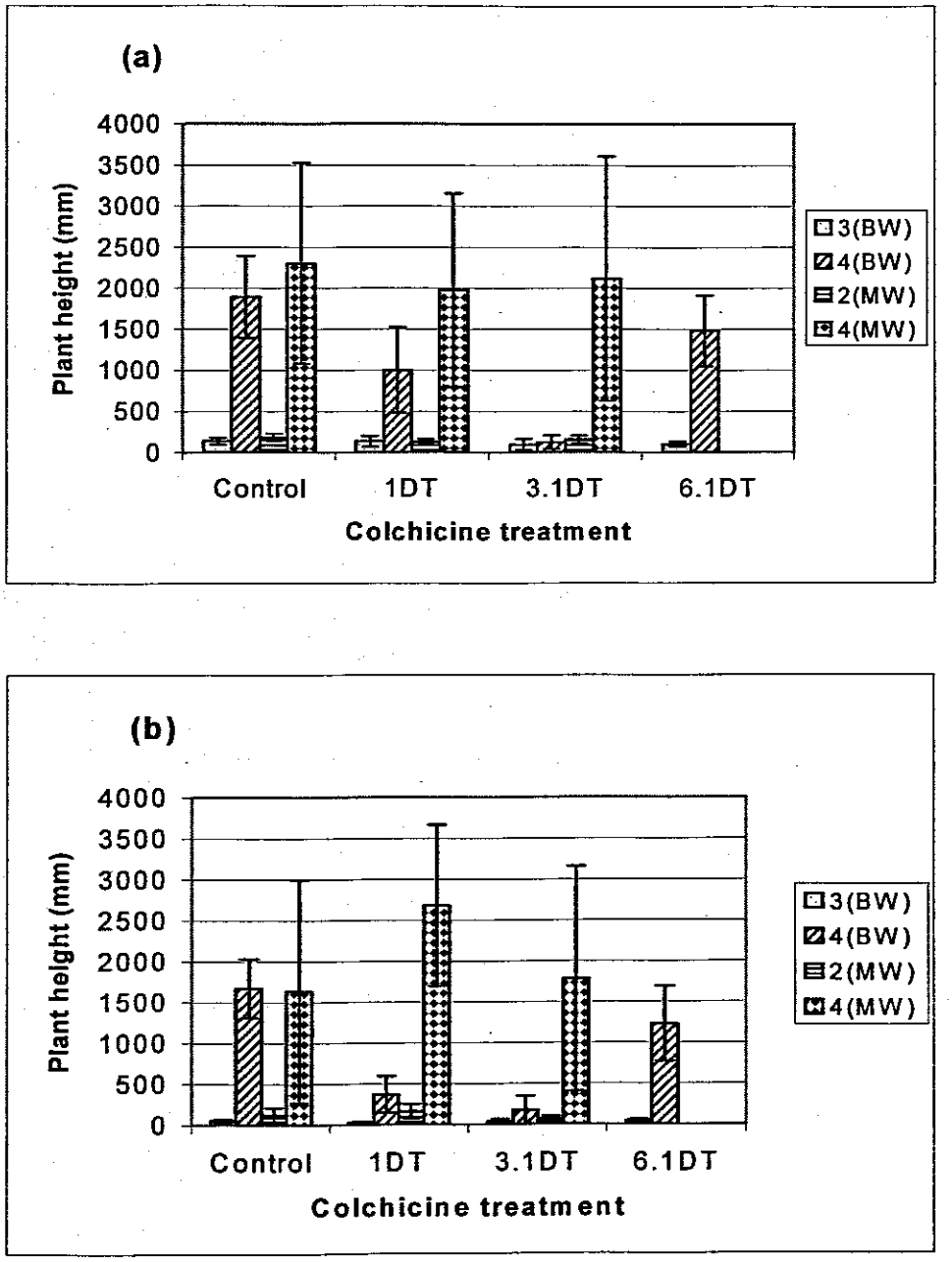


Figure 4.21: Effect of polyploidy on *C. palmata* (a) and *L. sphaerica* (b) plant height

Legend: Digit before the brackets: plant age in months; BW: plants grown in the beginning of winter (April); MW: plants grown in mid-winter (June).

Figure 4.21c *L. sphaerica* plants grown in the beginning of winter (4BW) showing the effect of colchicine treatment on plant height

Figure 4.21d: *C. palmata* plants grown in the beginning of winter (4BW) showing the effect of colchicine treatment on plant height.

ii) Effect of polyploidy on stem branching of *C. palmata* and *L. sphaerica*

The number of stems per plant was determined. It was noticed that as the untreated plants grew they sent off new shoots either near the soil level or somewhere along the initial stem (on leaf axis).

Table 4.19 (Appendix II) and Figure 4.22a show that all treated *C. palmata* plants grown in the beginning of winter did not show stem branching at the age of three months but after four months they all had an average of two stems per plant. The increase in the number of stems per plant in treated plants was not significant compared with the control. Results further indicate that branching rate of the control plants was slower than that of treated plants. As the plants grew from three to four months, all treated plants had an average of two stems per plants but control plants still had one.

Mid-winter grown and treated plants had an increased number of stems per plant at both two and four months of age but the increase was only significant at the age of two months. There was a significant increase in the number of stems per plant as plants grew from two to four months for control and 1DT plants.

Treated *L. sphaerica* (Table 4.19, Figure 4.22b) plants did not have any stem branching at both three and four months' age for plants grown in the beginning of winter. Treated plants grown in mid-winter had no stem branching at the age of two months but after four months there was stem branching for both control plants and treated plants. 1DT plants had a significantly lower number of stems per plant compared with the control. Both control and treated plants had an increased number of stems per plant after four months compared to after two months.

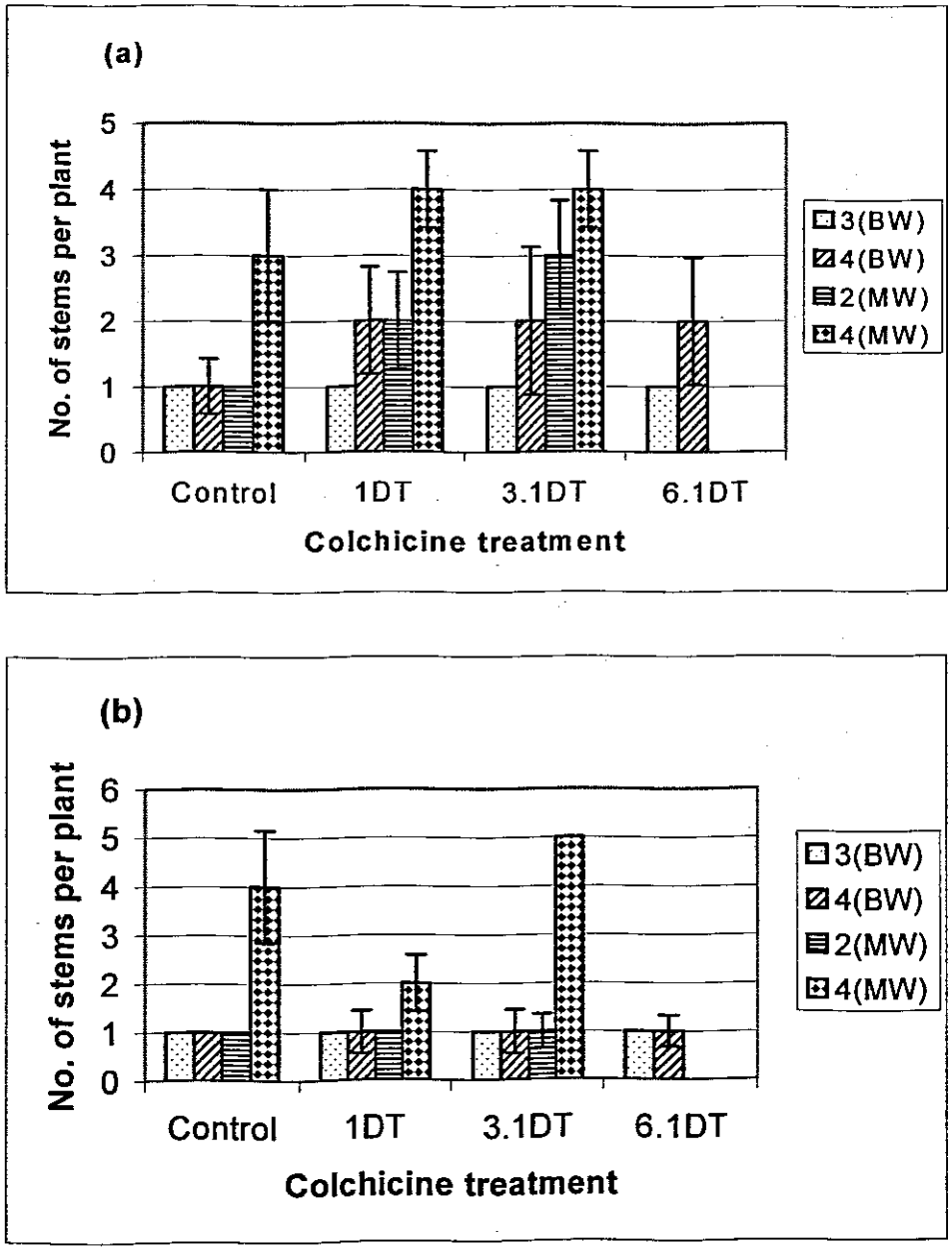


Figure 4.22: Effect of polyploidy on *C. palmata* (a) and *L. sphaerica* (b) stem branching

Legend: Digit before the brackets: plant age in months; BW: plants grown in the beginning of winter (April); MW: plants grown in mid-winter (June).

iii) Leaf analysis of *C. palmata* and *L. sphaerica*

The number of leaves per plant, leaf area, stomatal count and guard cell length were determined and the results appear in Table 4.19 (Appendix II) and Figure 4.23.

- **Number of leaves**

The average number of leaves per plant for all the plants per treatment was determined according to the method described in section 3.2.7.

Treated *C. palmata* plants grown in the beginning of winter (Figure 4.23a) did not differ from control plants after three months, all plants having six leaves each. After four months, however, there was a significant decrease for 3.1DT. Mid-winter grown control plants had a larger number of leaves after both two and four months compared to the plants grown in the beginning of winter. Similar results were found with *L. sphaerica*.

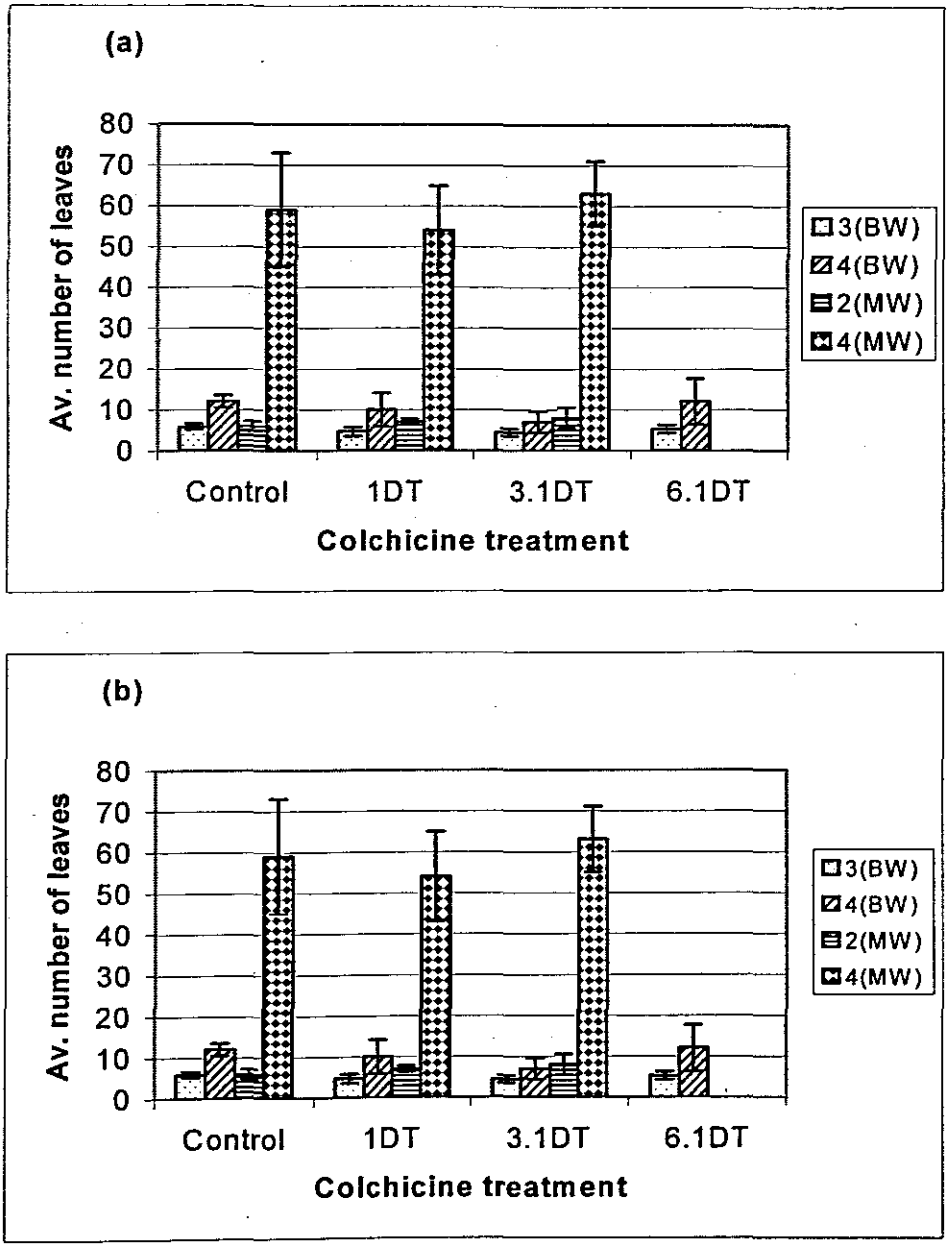


Figure 4.23: Effect of polyploidy on the number of leaves of *C. palmata* (a) and *L. sphaerica* (b)

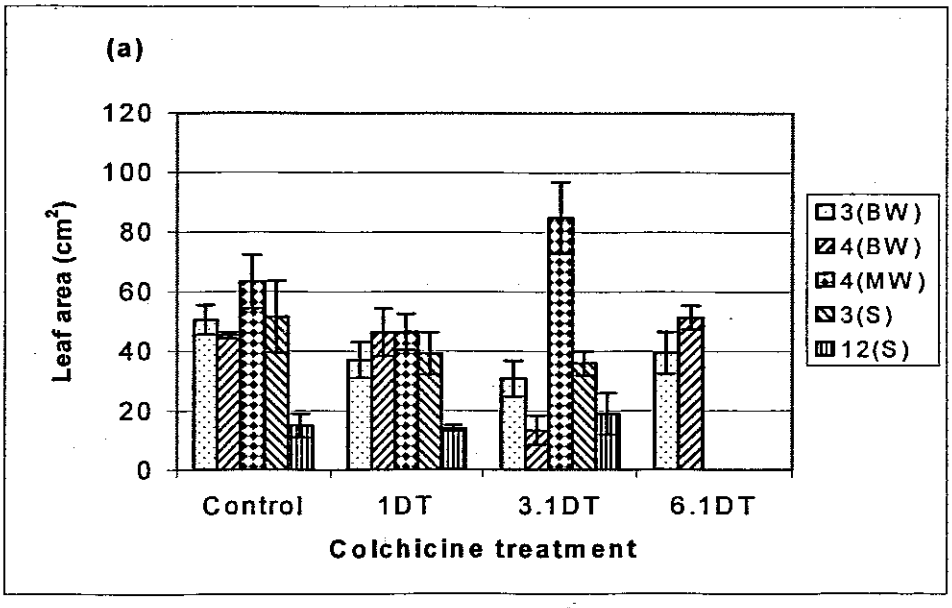
Legend: digit before the brackets: plant age in months; BW: plants grown in the beginning of winter (April); MW: plants grown in mid-winter (June).

- **Leaf area**

Leaf area (cm^2) was determined according to section 3.2.7. Results appear in Table 4.24 and Figure 4.24.

According to Figure 4.24a, no significant difference was found in leaf areas of beginning of winter (BW) and mid-winter (MW) grown control plants of both species. Summer grown control plants of both species (12S) had significantly smaller leaf areas. Both 1DT and 3.1DT *C. palmata* plants grown in the beginning of winter had significantly smaller leaf area after three months, and only 3.1DT after four months, compared to the control. Mid-winter grown 3.1DT plants had significantly larger leaf area compared to the control and 1DT. There was no significant difference in treated plants grown for both three (3S) and 12 (12S) months compared to the control.

Figure 4.24b indicates that both three and four month old *L. sphaerica*, treated plants grown in the beginning of winter had a significantly reduced leaf area compared to the control. Both mid-winter and summer grown treated plants did not have a significant difference in leaf area compared with the control.



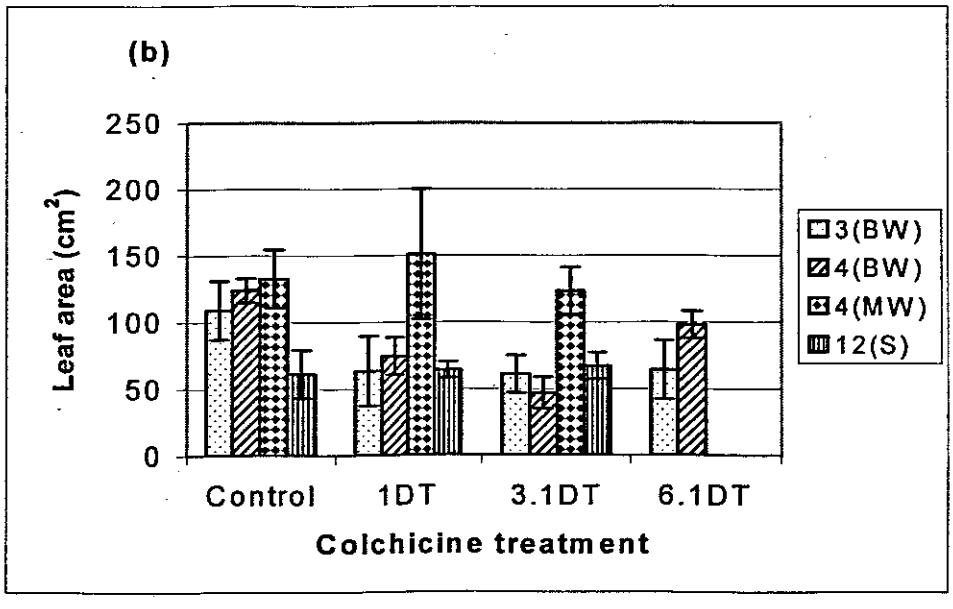


Figure 4.24: Effect of polyploidy on *C. palmata* (a) and *L. sphaerica* (b) leaf area

Legend: digit before the brackets: plant age in months; BW: plants grown in the beginning of winter (April); MW: plants grown in mid-winter (June); S: plants grown in summer (January)

- **Stomatal count and guard cell measurement**

Both *C. palmata* and *L. sphaerica* species have hypostomatic leaves (stomata occurring only on the lower epidermis) and the results refer to the number of stomata on the lower epidermis as described in section 3.2.7. Guard cell length was also measured according to section 3.2.7.

No treatment (1DT, 3.1DT nor 6.1DT) had a significant effect on the number of stomata of *C. palmata* when plants were grown in the beginning of winter (Figure 4.25). If the plants were grown in the summer, all the treatments (1DT and 3.1DT) showed a significant reduction in number of stomata. Similarly for *L. sphaerica*, 3.1DT showed a significant reduction in stomata, significantly for summer grown plants.

The results in Figure 4.26 indicate that three month old, summer grown, treated *C. palmata* plants showed no increase in guard cell length compared with the control. However, *L. sphaerica* treated plants showed a significant increase in their guard cell length, specifically at 3.1DT. Treatment affected the guard cell size of *L. sphaerica* but not that of *C. palmata*.

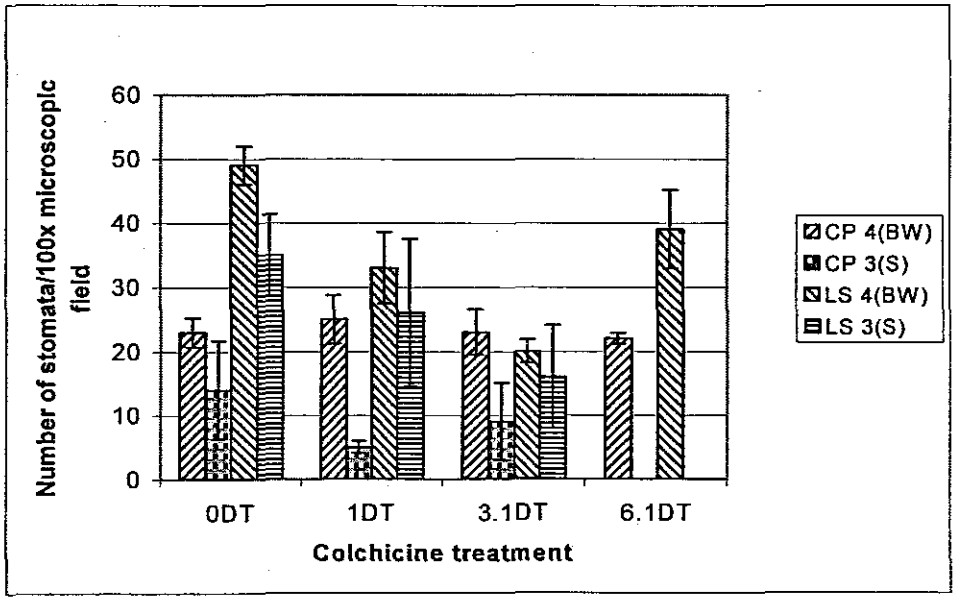


Figure 4.25: Effect of polyploidy on number of stomata in *C. palmata* and *L. sphaerica* leaves

CP: *C. palmata*; LS: *L. sphaerica*; digit before the brackets: plant age in months; BW: plants grown in the beginning of winter (April); S: plants grown in summer (January)

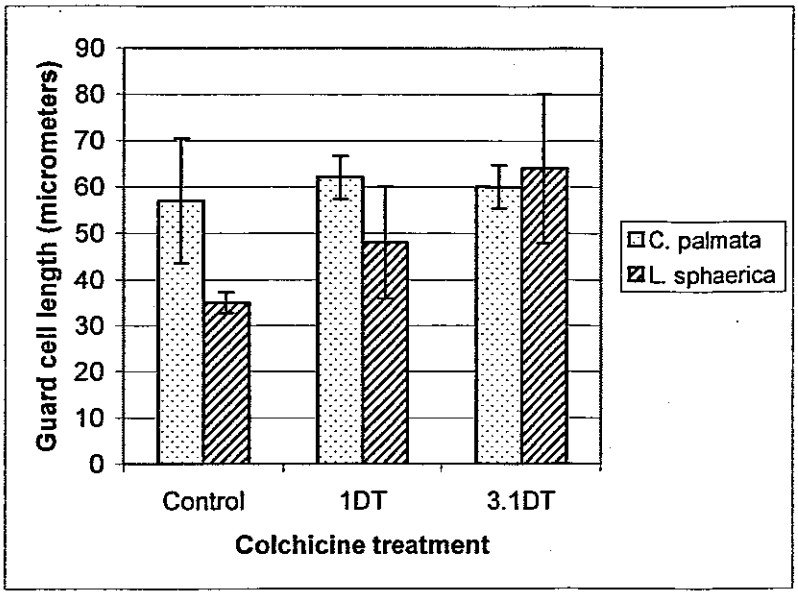


Figure 4.26: Effect of polyploidy on *C. palmata* and *L. sphaerica* guard cell length

4.5.3.3 Effect of polyploidy on plant physiology

The effect of polyploidy on the leaf chlorophyll, shoot and root fresh and dry weights, and percentage moisture content of both species, grown in the beginning of winter, mid-winter and summer was determined after a growth period ranging from two to ten months as described in section 3.2.9 (Table 4.19 (Appendix II) and Figure 4.27 to 4.34).

a) Fresh and dry weight

i) Shoot

The effect of polyploidy on fresh and dry weight (g) for the shoots (combination of leaves and stems) was determined per plant per treatment according to section 3.2.7. Results appear in Table 4.19 (Appendix II) and Figures 4.29 and 4.30.

Fresh and dry weight of both *C. palmata* (Figure 4.27a and 4.28a) and *L. sphaerica* (Figure 4.27b and 4.28b), when grown for three months in the beginning of winter, were significantly reduced for treatments 1DT and 3.1DT. This effect was still clear after four months, with 3.1DT still significantly lower. When grown in mid-winter, no significant effect of treatments on both species was found, except that 3.1DT had an initial (at 2 months) significant reduction in fresh weight for *L. sphaerica* (Figure 4.27b).

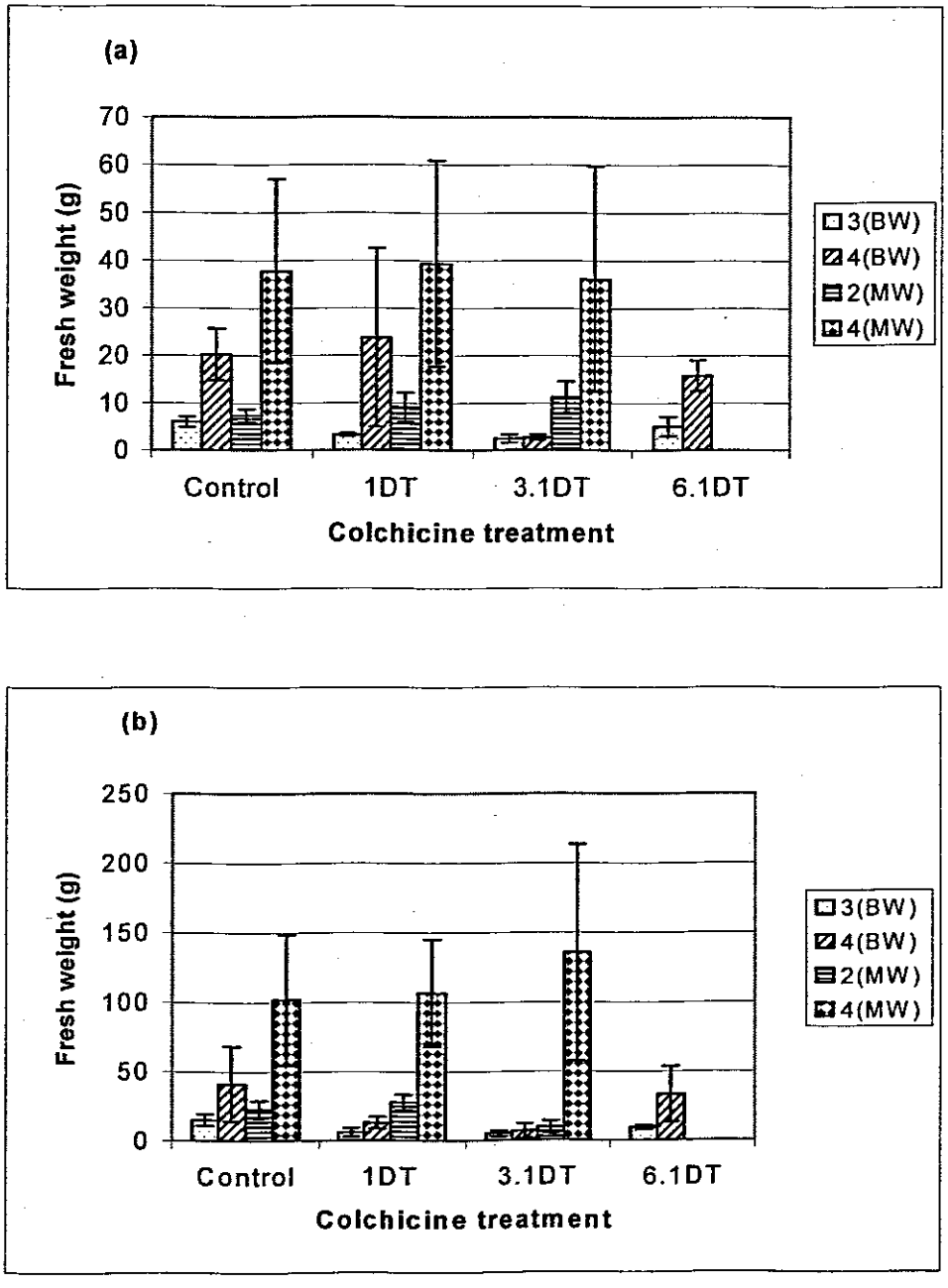


Figure 4.27: Effect of polyploidy on *C. palmata* (a) and *L. sphaerica* (b) shoot fresh weight

Legend: digit before the brackets: plant age in months; BW: plants grown in the beginning of winter (April); MW: plants grown in mid-winter (June).

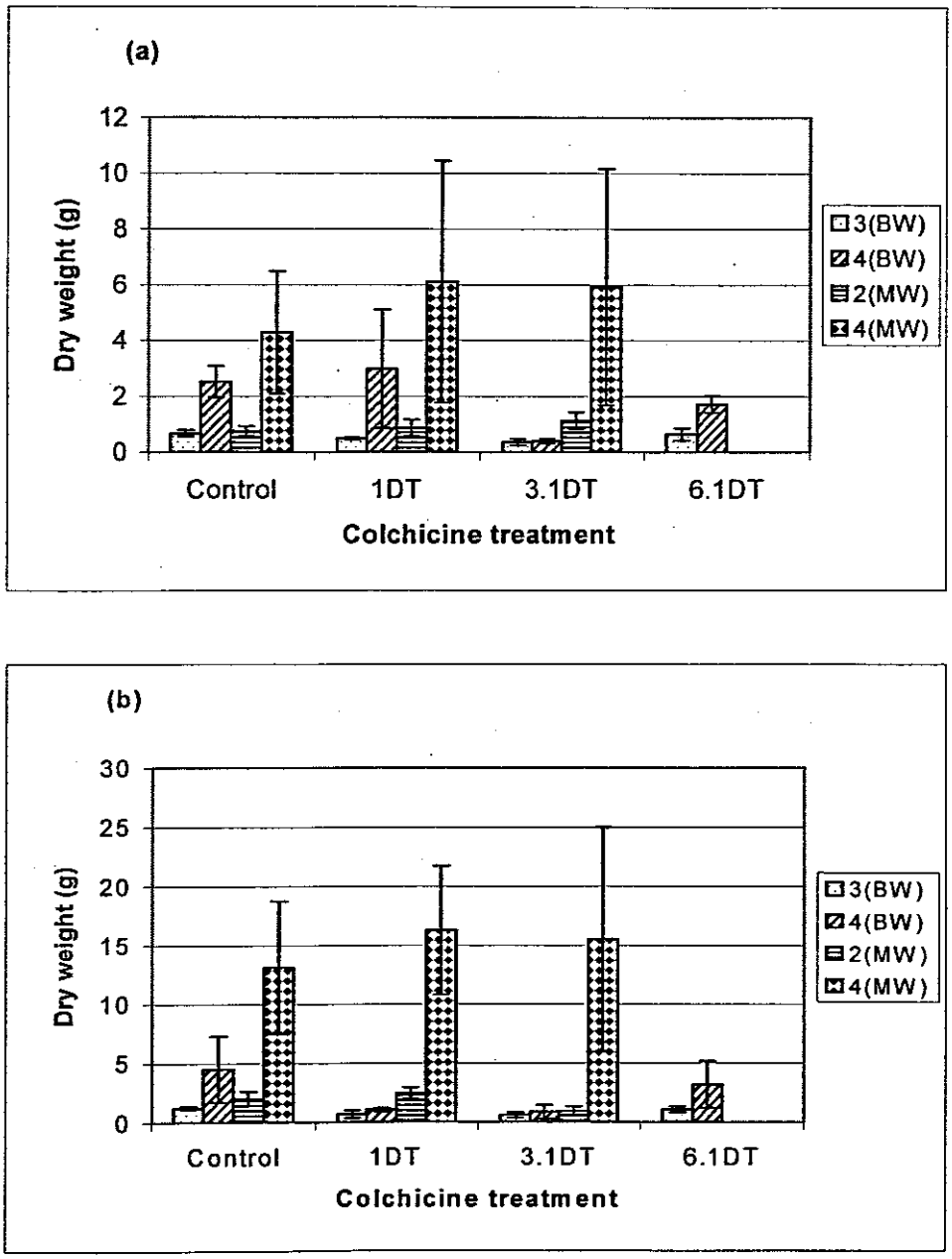


Figure 4.28: Effect of polyploidy on *C. palmata* (a) and *L. sphaerica* (b) shoot dry weight

Legend: digit before the brackets: plant age in months; BW: plants grown in the beginning of winter (April); MW: plants grown in mid-winter (June).

ii) Roots

C. palmata has bulbous roots which resemble the shape of a potato and *L. sphaerica* has fibrous roots. The effect of polyploidy on fresh and dry weight (g) on roots of *C. palmata* and *L. sphaerica* was determined, according to section 3.2.7. *L. sphaerica* roots (10BW and 8MW) were not analysed since they are fibrous and intertwine with one another. Results appear in Table 4.19 (Appendix II) and Figures 4.29 and 4.30.

Figures 4.29a and 4.30a indicate that during early stages of plant growth in the beginning of winter [3(BW) and 4(BW)] and mid-winter [2(MW) and 4(MW)], all treatments did not show a significant difference in fresh and dry weight of *C. palmata* roots compared with the control. However, after ten months for plants grown in the beginning of winter and eight months for plants grown in mid-winter, 1DT and 3.1DT resulted in a significant increase in fresh and dry weight, respectively.

In *L. sphaerica*, roots from plants grown in the beginning of winter, 1DT after three months and both 1DT and 3.1DT after four months, had a significant reduction in fresh and dry weight (Figure 4.29b and 4.30b) compared with the control. In mid-winter grown plants no treatments were significantly different from the control.

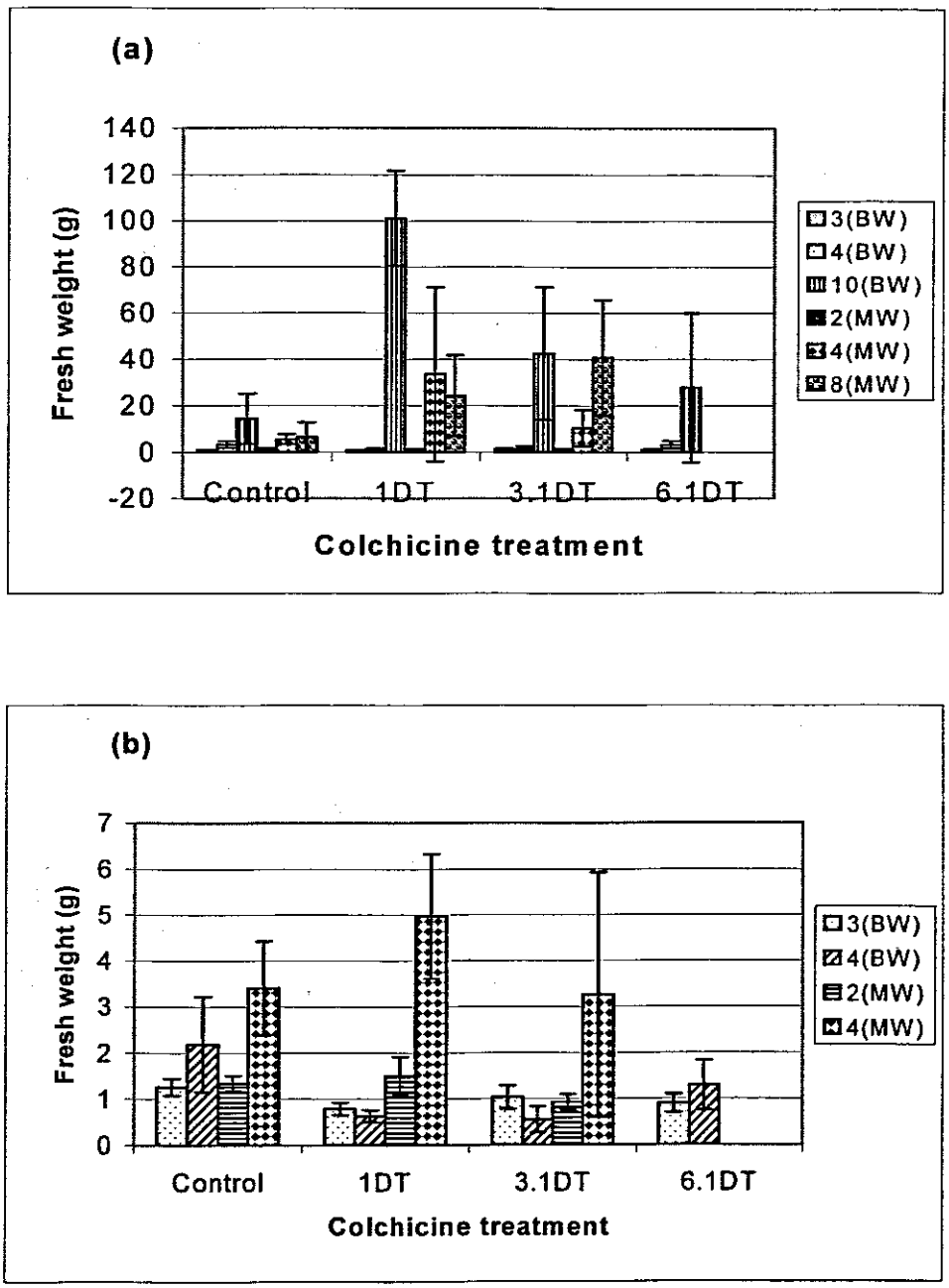


Figure 4.29: Effect of polyploidy on *C. palmata* (a) and *L. sphaerica* (b) root fresh weight

Legend: digit before the brackets: plant age in months; BW: plants grown in the beginning of winter (April); MW: plants grown in mid-winter (June).

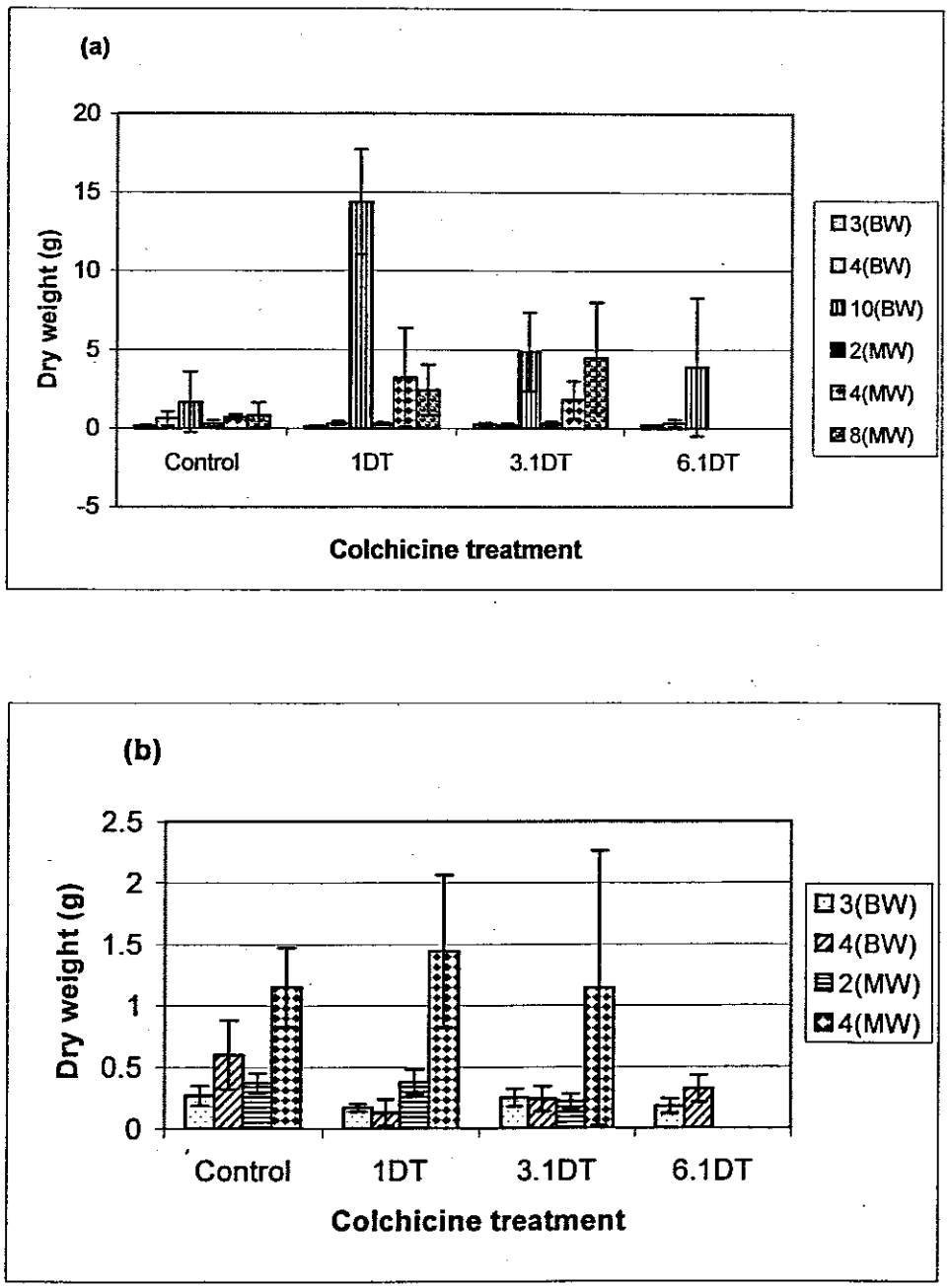


Figure 4.30: Effect of polyploidy on *C. palmata* (a) and *L. sphaerica* (b) root dry weight

Legend: digit before the brackets: plant age in months; BW: plants grown in the beginning of winter (April); MW: plants grown in mid-winter (June).

b) Moisture content

i) Shoots

Percentage moisture content is derived from the calculated fresh and dry weight of *C. palmata* and *L. sphaerica* according to section 3.2.7. Results appear in Table 4.19 (Appendix II) and Figure 4.31.

The 1DT and 3.1DT of both species showed a significant decrease in percentage moisture content after three months for plants grown in the beginning of winter. This effect started to disappear but after four months it was still seen for *C. palmata* (Figure 4.31a) at 3.1DT and for *L. sphaerica* (Figure 4.31b) at 1DT. Treatments of mid-winter grown plants had no effect after two months, but after four months percentage moisture content for 3.1DT was significantly lower for *C. palmata* and 1DT for *L. sphaerica*.

ii) Roots

The same method that was used to determine the percentage moisture content of the shoots was also used for roots (section 4.5.2.2 (d)). Results appear in Table 4.19 (Appendix II) and Figure 4.32a & b.

All treatments did not show a significant difference in root percentage moisture content in *C. palmata* and *L. sphaerica* plants grown in the beginning of winter and mid-winter. However, only 3.1DT showed significantly lower percentage moisture content of *L. sphaerica* roots grown in the beginning of winter, after four months.

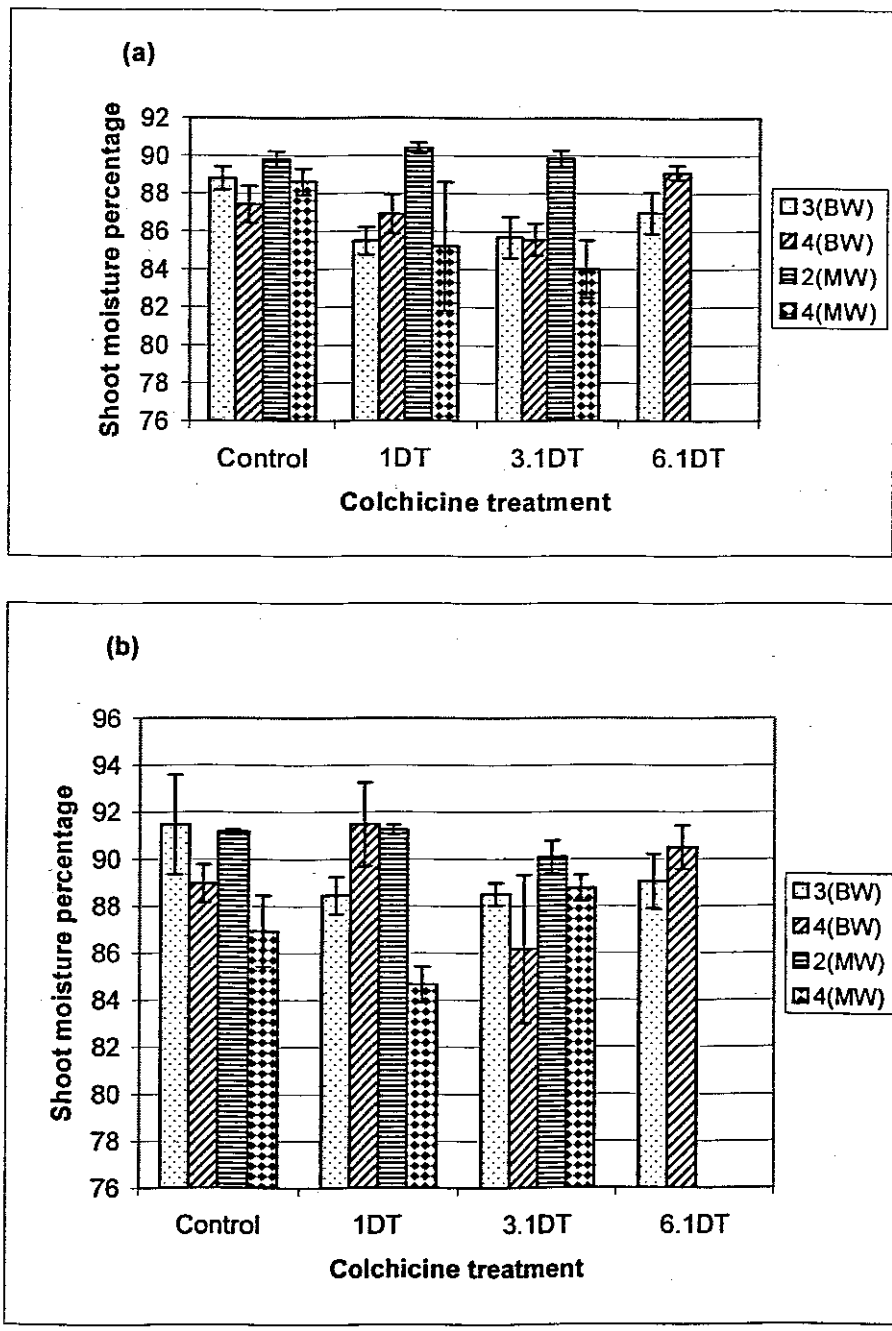


Figure 4.31: Effect of polyploidy on percentage moisture content of *C. palmata* (a) and *L. sphaerica* (b) shoots

Legend: digit before the brackets: plant age in months; BW: plants grown in the beginning of winter (April); MW: plants grown in mid-winter (June).

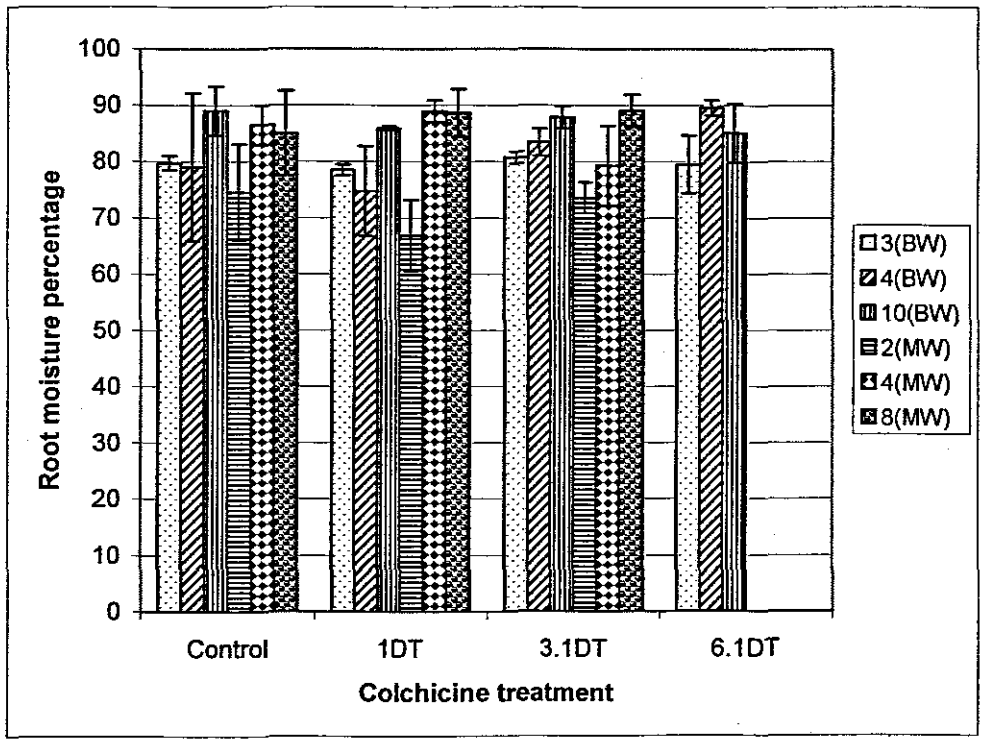


Figure 4.32a: Effect of polyploidy on percentage moisture content of *C. palmata* roots

Legend: digit before the brackets: plant age in months; BW: plants grown in the beginning of winter (April); MW: plants grown in mid-winter (June).

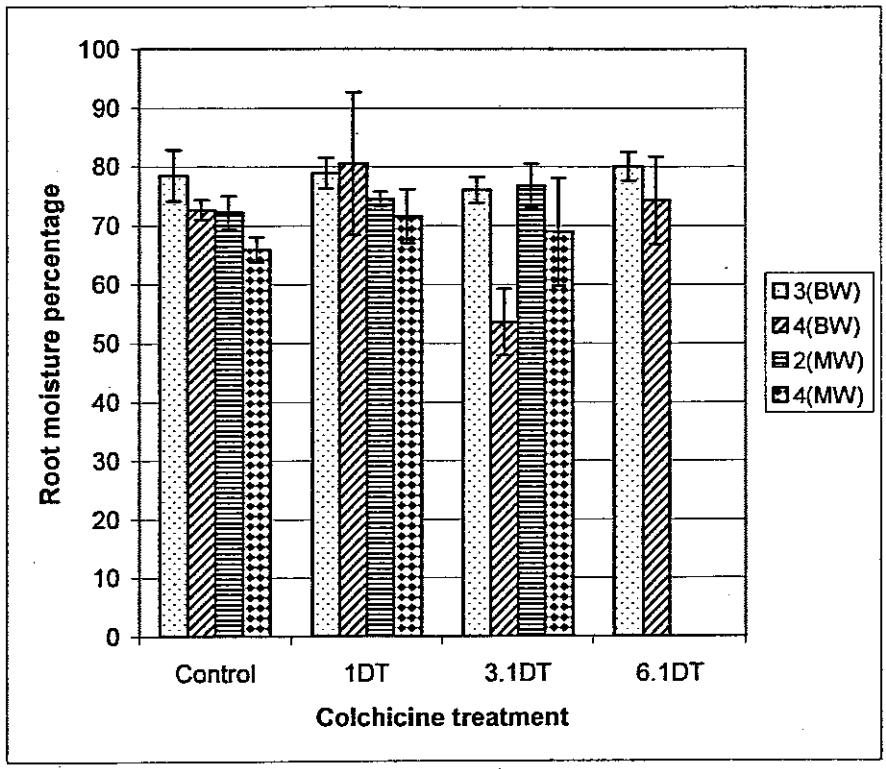


Figure 4.32b: Effect of polyploidy on percentage moisture content of *L. sphaerica* roots

Legend: digit before the brackets: plant age in months; BW: plants grown in the beginning of winter (April); MW: plants grown in mid-winter (June).

c) Leaf chlorophyll content

i) 90% Acetone extraction

Chlorophyll extraction with acetone from 12 month old leaves was preceded with grinding in contrast to the extractions done in earlier plant age, where no grinding of leaves was applied.

After three months growth of treated, beginning of winter grown plants, the chlorophyll content of *C. palmata* was significantly increased by 1DT and 3.1DT treatment (Figure 4.33a), but for *L. sphaerica*, a similar effect was shown by 3.1DT and 6.1DT treatments (Figure 4.33b). After four months growth this effect was still clear with 3.1DT and 6.1DT for *C. palmata*, as well as 1DT and 6.1DT for *L. sphaerica*. A similar response was found with mid-winter grown plants for both species, where 3.1DT (*C. palmata*) and 1DT, 3.1DT (*L. sphaerica*) caused significant increases in chlorophyll content. This is, however, not the case with summer grown plants of both species because no treatment had any effect on chlorophyll content after three and 12 months growth.

ii) Dimethyl Sulphoxide (DMSO) extraction

The leaf chlorophyll extraction was from three and five month old, mid-winter grown plants. At the age of five months, plants for both species were having many yellow leaves marking the decline in their growth.

Figure 4.34 indicates that after three months the chlorophyll content of *C. palmata* leaves was significantly reduced by 1DT and 3.1DT and significantly increased by 3.1DT after five months. In response to the growth declining period (indicated by yellowing of some leaves), control plants had significantly reduced chlorophyll content after five months compared to three months, but treated

plants did not. Colchicine treatment caused no significant difference in leaf chlorophyll content of *L. sphaerica* after three and five months. However, 1DT showed a significant reduction in chlorophyll content after five months compared to three months.

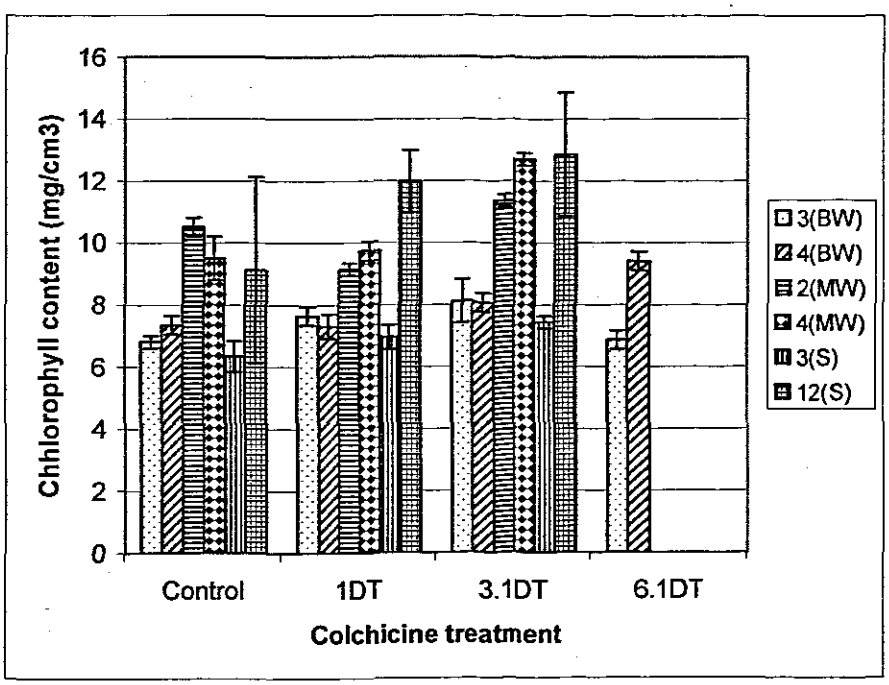


Figure 4.33a: Effect of polyploidy on leaf chlorophyll content of *C. palmata* using 90% Acetone

Legend: digit before the brackets: plant age in months; BW: plants grown in the beginning of winter (April); MW: plants grown in mid-winter (June).

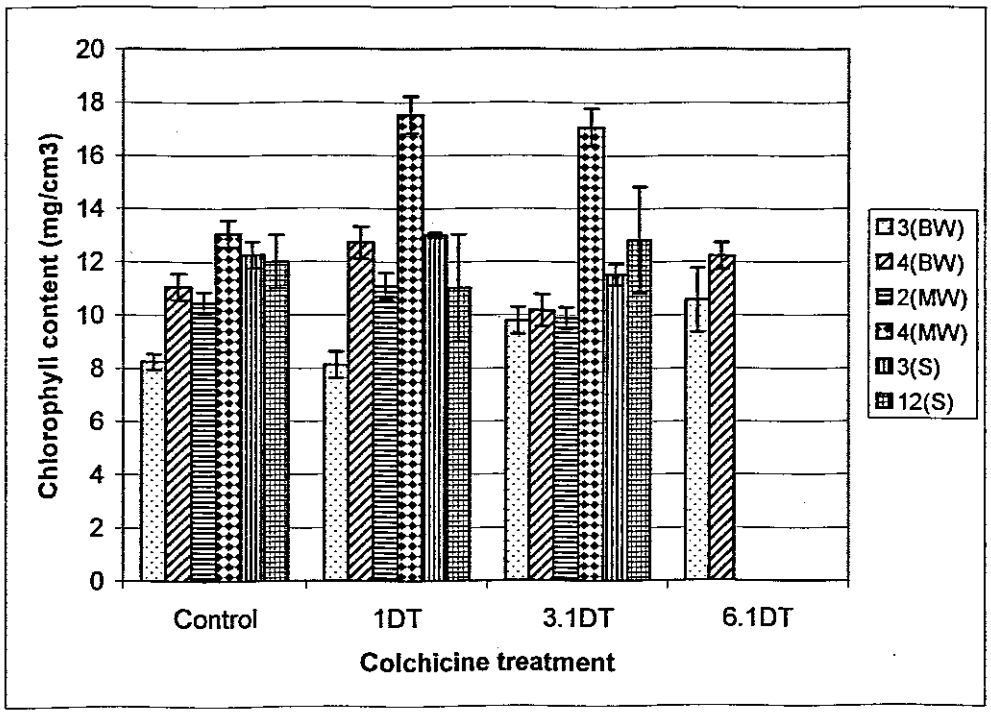


Figure 4.33b: Effect of polyploidy on leaf chlorophyll content of *L. sphaerica* using 90% Acetone

Legend: digit before the brackets: plant age in months; BW: plants grown in the beginning of winter (April); MW: plants grown in mid-winter (June).

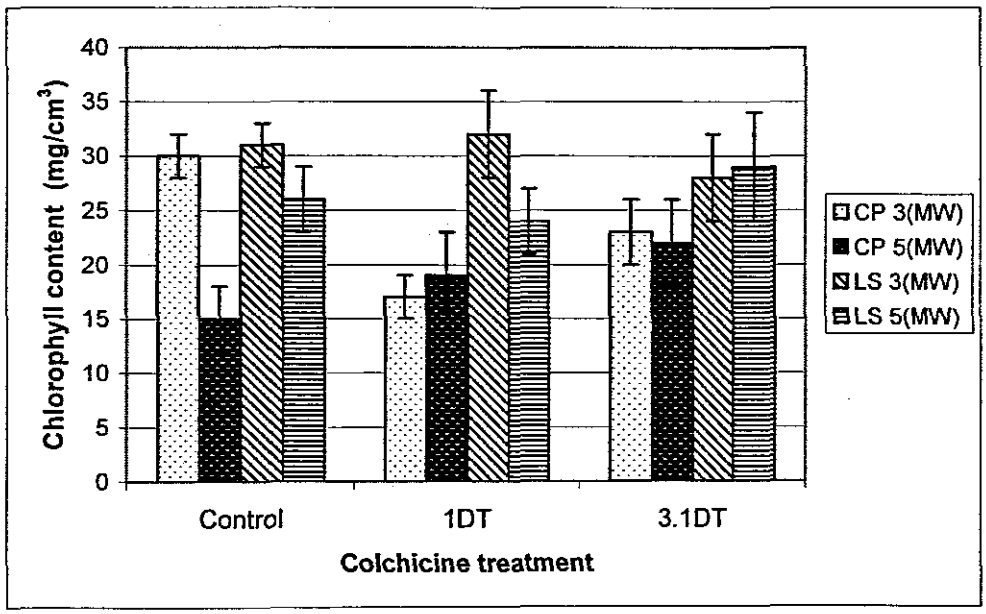


Figure 4.34: Effect of polyploidy on leaf chlorophyll content of *C. palmata* and *L. sphaerica* using DMSO

Legend: CP: *C. palmata*; LS: *L. sphaerica*; digit before the brackets: plant age in months; BW: plants grown in the beginning of winter (April); MW: plants grown in mid-winter (June).

4.5.3.4 Effect of polyploidy on the phenology of *C. palmata* and *L. sphaerica* species

Phenology is the study of events taking place in the plant from seed germination, seedling and plant growth, plant reproduction and death. The phenology of untreated *C. palmata* (collected at Eshowe) and *L. sphaerica* (KwaDlangezwa Cross Roads) was first studied in the wild where seeds were collected (Table 4.20). Only the vegetative, flowering, fruiting and declining stages were observed in the plants growing in the wild.

Table 4.20: Phenology of *C. palmata* and *L. sphaerica* species in the wild

Phenology event	*C. palmata*	*L. sphaerica*
Vegetative growth	August – December	July/August – October
Flowering period	September – March	August – June
Fruiting period	November – June	September – May
Declining stage	April - August	February – August

The results, as indicated in Table 4.20, show that *C. palmata* grows in spring to summer and the species flowers and bear fruits from summer to autumn. When the plants are in their fruiting stage, the shoots start drying out and then die but the roots (bulbs) remain underground and send new shoots out during the following spring.

L. sphaerica plants also grow in spring to summer. These plants flower almost throughout the year. It was, however, noted that there are many flowers found in spring to summer compared with autumn to winter. Fruit initiation normally starts soon after the flowering stage has started. There is a very high mixture of flowering and fruiting stages in this species. It was observed that flowers do not

open all at once but rather flowering is a continuous process. Fruiting follows the same trend. Drying of the shoots also takes place when most of the fruits that were initially set are ripe.

a) Effect of polyploidy on plants grown below 400µmole/m^2/s light intensity in the nursery

The results obtained from the effect of light intensity on plant growth (section 4.5.1) indicate that a maximum of 400 µmole/m^2/s light intensity be selected for further growth of plants under nursery conditions. Radicle emergence and the first leaf formation were included for phenology investigation. Results for plants grown in the beginning of winter and mid-winter appear in Table 4.21.

Both diploid and polyploid plants showed the same results as indicated in the table. *C. palmata* started germinating on day four up to day ten while *L. sphaerica* started on day three to day ten. *L. sphaerica* started leaf formation slightly earlier (day 13) than *C. palmata* (day 14). Nursery-grown plants did not bear flowers and therefore no fruits developed. After five months of growth, *L. sphaerica* started to show yellow leaves that gradually led to the drying of the shoots, which extended up to seven months. For *C. palmata* plants, leaf yellowing was initiated slightly later (six months).

New shoots were sent out from the root stalk of both plants after their growth declined. It was then assumed that shade conditions promote plant rejuvenation rather than maturity. To continue with the investigation, plants were grown under different shade conditions (light intensities). The further aspect of pot effect was investigated by growing plants with bags and without bags as explained in the following section.

Table 4.21: Effect of polyploidy on the phenology of *C. palmata* and *L. sphaerica* in the nursery at 400 µmole/m^2/s light intensity (with control, 1DT and 3.1DT plants)

Phenology Event	*C. palmata*	*L. sphaerica*
Radicle emergence	Day 4-12	Day 3-10
First leaf formation	Day 14-18	Day 13-18
Flower onset	-	-
Fruit onset	-	-
Fruit ripening	-	-
Decline (drying of foliage)	6-7 months	5-7 months

b) Effect of polyploidy, light intensity and plant bag on *C. palmata* and *L. sphaerica* plant morphology

Plants from seedling trays were initially hardened at <300 µmole/m^2/s light intensity before being transferred to respective light intensities at day nought and after four and eight weeks. Plants were grown in pots and plastic bags in the nursery at <300; 800 and >1000 µmole/m^2/s light intensities.

C. palmata plants were transferred from the nursery (<300 µmole/m^2/s light intensity) to the soil (>1000 µmole/m^2/s light intensity), with and without bags at the same time intervals as previously mentioned, but *L. sphaerica* was transferred to the soil on day nought without bags due to the very low seedling quantity after virus infection. As a result, *L. sphaerica* results shown are for >1000 µmole/m^2/s light intensity in the nursery and in the field without bags at day nought transferral.

(i) Nursery grown plants

Results in Table 4.22 show that colchicine treatment did not have any effect on phenological events of both species compared with the control.

Table 4.22: Effect of polyploidy and light intensity on *C. palmata* and *L. sphaerica* nursery grown plants

Event	*L. sphaerica*			*C. palmata*		
	control	1DT	3.1DT	control	1DT	3.1DT
Radicle emergence	Day 3-10	Day 3-10	Day 3-10	Day 4-11	Day 4-11	Day 4-11
First leaf formation	Day 13-17	Day 13-17	Day 13-17	Day 14-18	Day 14-18	Day 14-18
Flower onset	-	-	-	-	-	-
Fruit onset	-	-	-	-	-	-
Fruit ripening	-	-	-	-	-	-
Decline (foliage drying)	5-6 months	5-6 months	5-6 months	5-7 months	5-7 months	5-7 months

Legend: One day seed treatment before (1DT) and after three (3.1DT) days incubation

(ii) Field grown plants

According to Table 4.23, colchicine treatment did not cause any difference in radicle emergence and first leaf formation in both species compared with the control. *C. palmata* plants grown in the soil with bags earlier (3 months from incubation) started dying later (after a month) compared to those grown later (4 and 5 months from incubation) that started dying after two weeks. *C. palmata* plants grown in soil without bags gave rise to male flowers only, except plants from 3.1DT, transferred after five months from incubation, which did not flower.

All *L. sphaerica* plants were grown in the soil three months from incubation, without bags. Control plants gave male flowers before treated plants (7 months from incubation compared to 7.5 months from incubation). Only control plants had female flowers that led to fruiting. After 11 months, both *C. palmata* and *L. sphaerica* foliage started drying.

Table 4.23: Effect of polyploidy and growth in plant bag on the phenology of *C. palmata* and *L. sphaerica* plants transferred to the soil at different time intervals

Time	*C. palmata*																		*L. sphaerica*		
	with bag			in soil			with bag			in soil			with bag			in soil			in soil		
	cont	1DT	3.1 DT	cont	1DT	3.1 DT	cont	1DT	3.1 DT	cont	1DT	3.1 DT	cont	1DT	3.1 DT	cont	1DT	3.1 DT	cont	1DT	3.1 DT
D 0	SI	SI	SI	SI	SI	SI	SI	SI	SI	SI	SI	SI	SI	SI	SI	SI	SI	SI	SI	SI	SI
D 3-10																			RE	RE	RE
D 4-10	RE	RE	RE	RE	RE	RE	RE	RE	RE	RE	RE	RE	RE	RE	RE	RE	RE	RE			
D 13-17																			L1	L1	L1
D 14-18	L1	L1	L1	L1	L1	L1	L1	L1	L1	L1	L1	L1	L1	L1	L1	L1	L1	L1			
M3	T	T	T	T	T	T													T	T	T
M4	De	De	De				T	T	T	T	T	T									
M 4.5	De	De	De				De	De	De												
M5	De	De	De				De	De	De				T	T	T	T	T	T			
M 5.5	De	De	De				De	De	De				De	De	De						
M6	De	De	De				De	De	De				De	De	De						
M7							De	De	De				De	De	De				Ma		
M 7.5													De	De	De				F	Ma	Ma
M8													De	De	De				Fr		
M9				Ma								Ma				Ma					
M 9.5						Ma													Frr		
M 10					Ma						Ma						Ma		Frr		
M 10.5										Ma									Frr		
M 11				De	De	De				De	De	De				De	De	De	Frr & De	De	De
M 12				De	De	De				De	De	De				De	De	De	De	De	De

Legend: **D:** day, **M:** month, **cont.:** control, one day seed treatment before (**1DT**) and after three days (**3.1DT**) incubation, **SI:** seed incubation, **RE:** radicle emergence, **L1:** first leaf formation, **T:** transference to soil (with and without bags), **F:** female flower onset, **Ma:** male flower onset, **Fr:** fruit onset, **Frr:** fruit ripening, **De:** decline (foliage drying)

4.5.3.5 Discussion

(a) Polyploidy effect on morphology and physiology

The effect of polyploid induction on the morphology (stem height and branching, and leaves) and physiology (chlorophyll production, shoot and root weights and percentage moisture content) on both species was investigated.

The 1DT and 3.1DT plants showed significant reduction in plant height, and fresh and dry weight shoots of both species, but showed significant increase in the fresh and dry weight of the roots, with biggest effect in *C. palmata*. These results correspond with that of Hannweg and Vos (2001) and Beck *et al.* (2003), that polyploidy can induce dwarfism in plants. Tilney-Bassett (1986) also reports that shoot growth is retarded, stunted and twisted, for example in *Amaranthus*, after the induction of polyploidy with colchicine. Again, Gao *et al.* (1996) reports that polyploid plants are vigorously growing plants compared to their diploid associates.

The reduction in plant height was accompanied by the branching phenomenon of the treated plants. Treated *C. palmata* plant stems, grown in mid-winter, branched after two and four months and those grown in the beginning of winter branched after four months. The *L. sphaerica* treated stems branched only after four months for mid-winter grown plants.

Results for both species revealed that, as the plant elongated there was a small increase in the number of leaves formed and only the internodes were elongated. This sometimes caused dwarf plants to have many leaves rather than giant ones. The stem branching also increased the surface area for leaf development in dwarf plants. The above statement is perhaps misleading for very short plants because they yield a very small number of leaves. Therefore,

the use of number of leaves per plant criterion was not effective in investigating the influence of chromosome doubling in plants.

Colchicine treatment resulted in no significant difference in shoot and root percentage moisture content of both species.

According to the results obtained, colchicine treatment showed reduction in the leaf area of both species. This aspect, however, resulted in a some fluctuations. The leaf area can be affected by other factors such as growth rate and seasonal changes. Since the criterion is leaf position (leaf 8-10), leaf area measurement of vigorously growing control plants may be on juvenile leaves, thus causing a decrease in their leaf area.

Again, the reduction in leaf area may be due to the adaptation of treated plants to drought (in the beginning of winter), by reducing the surface area for transpiration. As Tilney-Bassett (1986) and Hannweg and Vos (2001) have reported, polyploid plants may have improved drought tolerance. However, as the summer begins (in mid-winter grown plants), the leaf area of the treated plants changes and become bigger than that of the control, indicating that when the drought season is over, polyploid plants switch over and start producing leaves with a bigger leaf area compared to the control (Gao *et al.* (1996).

Colchicine treatment resulted in the reduction in the number of stomata of both species compared with the control, with the greatest effect at 3.1DT. Again, both species had an increase in the size (length) of the guard cells, even though it was not significant for *C. palmata*. This corresponds to the findings of Tilney-Bassett (1986), Takamura and Miyajima (1996) and Beck *et al.* (2003) that tetraploid leaves have larger guard cell size and fewer stomata than their diploid relatives.

Initially, the chlorophyll extraction was done by using 90% Acetone. The leaves produced from plants with one day's treatment before germination (1DT) and one day's treatment after three days of incubation (3.1DT) showed some green, unextracted patches on leaf discs. It was thus decided necessary to adopt another chlorophyll extracting method to compare with. The method using dimethyl sulphoxide (DMSO) was used. According to Richardson *et al.* (2002), the chlorophyll extracts are more stable in DMSO and do not break down as quickly as those in acetone. Another advantage was the heating procedure in the DMSO method compared to storage in the fridge (cold room) in the acetone method.

L. sphaerica plants were highly affected by Cucumber Mosaic Virus (CMV) compared to *C. palmata* plants. This virus mostly causes leaf deformation as evidenced by the presence of wrinkled curled leaves. Virus-infected leaves normally wilt and drop before reaching maturity. The colchicine-treated *L. sphaerica* plants (especially 3.1DT) were highly affected compared with the control. Therefore, summer grown 3.1DT plants had re-establishing leaves at the age of four months since all leaves had fallen off previously. These re-establishing leaves made chlorophyll content results not comparable to the plants with mature leaves.

Acetone extraction indicated that colchicine treatment resulted in an increase in leaf chlorophyll content of the beginning of winter, and of mid-winter grown plants of both species. After four months, treated (1DT and 3.1DT) plants grown in the beginning of winter and mid-winter had leaf discs for both plants that were not fully extracted. Polyploidy may cause chlorophyll to be highly intact in the plastids as the plant grows, so that it cannot be easily extracted, and thus it becomes stress tolerant. These effects correspond to the findings of Hannweg and Vos (2001) and Martelotto *et al.* (2005) that polyploid plants have improved phosynthetic capacity and drought stress resistance/tolerance, and of Tilney-Bassett (1986) that diploid have normal green tissues and darker green

tetraploid tissues. Tilney-Bassett (1986) findings is confirmed in this investigation by four month old mid-winter grown treated plants that were in their growth decline stage but maintained high chlorophyll content even though there were unextracted patches in their leaf discs.

The results obtained from the use of DMSO confirm the stability in chlorophyll content of treated leaves during the growth decline stages, especially for *C. palmata* leaves. At the age of five months, plants were yellowing and the leaves were dying but the chlorophyll content were maintained as high as they were at three months of age. During chlorophyll extraction 1DT had some green patches that were remaining on leaves. Even when the heating period was increased to a maximum of 50 minutes, the patches could not be totally extracted which can be a good sign of increased stress tolerance in polyploids.

In the case of *L. sphaerica*, there is remarkable degree of stress tolerance for treated leaves induced by 3.1DT method. Even though the chlorophyll content seemed to be lower than that of the control leaves in the three month period, the advantage is that the treated leaves maintained that level under stress conditions (showed in chlorophyll extraction results at the age of five months).

The results confirmed that the chlorophyll extracts are more stable in DMSO and do not break down as quickly as in acetone (Richardson *et al.* 2002), since acetone had chlorophyll content ranging from 6-18mg/cm^3 but 15-29mg/cm^3 in DMSO for both species.

(b) Polyploidy effect on phenology

The effect of polyploidy induction on phenology (radicle emergence, first leaf formation, flowering, fruiting and growth decline) in both species was investigated. The Cucurbitaceae has an inferior ovary and therefore the fruit onset can only be considered at the fall of the corolla. Furthermore, it is difficult

to notice the fruit ripeness in *L. sphaerica* because the rind stays green even when the flesh is ripe. Again, the hardness of the rind does not necessarily or always means that the fruit is ripe inside.

In the wild, *L. sphaerica* had mixed vegetative, flowering and fruiting periods with a range of flower buds, mature flowers, immature and mature fruits at the same time. Flowering begins in summer and lasts until winter. Winter flowering yields small fruits followed by a complete disappearance of plant. Plants may flower more than once before shoot drying.

The *C. palmata* plants flowered in summer, soon after their growth stage that starts in spring. Soon after the initiation of fruit ripening, shoots die. As perennials, both species have their shoots drying and re-sprouting from the bulbous roots (*C. palmata*) and root stalks (*L. sphaerica*) the next spring.

Growth of both plant species in pots or bags, in the nursery, and at different light intensities inhibits plant maturity (flowering and fruiting) and promotes plant rejuvenation through re-sprouting of dried shoots from root stalk. Growth of plants in soil with bags at light intensity >1000µmole/m2/s also inhibited flowering in *C. palmata* and the surviving plants were stunted and died earlier (2 weeks to a month from transferral) compared to plants without bags (6-8 months after transferral). Plants in bags were root-bound and were unable to survive. Plant bags also increased the temperature around the roots and therefore enhanced the probability of plant death.

Both control and treated *C. palmata* plants grown without bags had male flowers, except for 3.1DT transferred after five months from incubation. Colchicine treatment inhibited female plant flowering in *L. sphaerica* and this may be because of reduction in plant fertility or semi-sterility caused by polyploidy (Tilney-Bassett 1986, Snyder *et al.* 1985, Gao *et al.* 1996).

CHAPTER FIVE

5.1 Summary and Conclusion

5.1.1 Summary

The northern-coastal and the mid-region of KwaZulu-Natal, South Africa, were used for specimen and seed collection purposes. This region includes coastal areas such as Mtunzini, Empangeni, Richards Bay, Nseleni and Mtubatuba, while the mid-region areas were Eshowe and Nkandla. From these areas, nine genera and eleven indigenous species were identified. About 50 specimens were collected, identified and prepared for herbarium purposes.

Among the collected Cucurbitaceae species, those to be used for study purposes were selected on the basis of their food and medicinal usage. Further, the selection criterion was the availability of sufficient seeds. Four species, namely, *Coccinia palmata* (Sond.) Cogn., *L. sphaerica* (Sond.) Naud., *Momordica balsamina* L. and *M. foetida* Schumach were selected for germination study. Both *Momordica* species did not germinate successfully under laboratory conditions and were thus excluded from the investigations.

The *C. palmata* occurs mostly in places with lower temperature and humidity. They usually occur on margins of thick forests and grow towards shady places (they are shade-loving plants). The *L. sphaerica* normally grows in a range from shady places through to full sunlight as they occur in forest margins, amongst shrubs and in grasslands. However, as climbers, both plants like climbing on trees and shrubs and have their fruits hanging, the fruits being shaded by their own or other plants' leaves.

Germination studies showed that both *C. palmata* and *L. sphaerica* germinated successfully at 25°C under light conditions. The germinated seedlings were

transferred to seedling trays with potting soil and were established or hardened in the nursery at light intensity <300µmole/m^2/s for a minimum of ten days. Hardened seedlings were thereafter transferred to 20l plant pots or 10l plant bags, or direct to the soil.

Plants grown at different light intensities showed an increase in plant height or length, leaf area and shoot fresh weight with a decrease in light intensity. However, at very low and high light intensities, root fresh and dry weight decreased for both species.

Polyploidy was induced by treating seeds with different colchicine concentrations ranging from 0.001g/l to 0.1g/l, before and after their incubation for germination, and at different time intervals (days). Seed treatment before incubation for germination induced sterile polyploids (triploids, pentaploids and heptoploids), especially in leaves of both species. Colchicine treatment after incubation for germination induced fewer sterile polyploids than treatment before incubation for germination. The longer (more than a day) exposure of seeds/seedlings to colchicine treatment and high colchicine concentrations (more than 0.01g/l) killed some cells within the plant tissue and also the seedlings.

After a series of experiments, as described in chapter 4 (section 4.3), using flow cytometry analysis, one day treatment before (1DT) and after three days (3.1DT) incubation for germination at 0.01g/l colchicine concentration, was used throughout the investigation. Colchicine treatment lowered the seed germination percentage in both methods by killing cells of germinating seeds.

Control and treated seedlings grown below 400µmole/m^2/s inhibited flowering and promoted plant rejuvenation. Grown plants had their shoots drying (dying) after seven months and later re-sprouting from the root stalk. Plants grown in full sun (>1000µmole/m^2/s) in the plant bags died at a high rate soon after their transplantation. The plant bags or pots increased the temperature of the roots

and thereafter killed the plants. Plants from treated seeds grown in the soil also had flowering inhibited compared to control plants. Colchicine treatment might have induced sterility in these plant species. Treated plants were more susceptible to Cucumber Mosaic Virus (CMV), especially 3.1DT plants compared to control plants.

Flow cytometry analysis indicated that both *C. palmata* and *L. sphaerica* had a higher rate of mitosis in roots than in leaves. Colchicine treatment induced tetraploid and even octoploids cells in leaves. However, some triploids, pentaploids and hexaploids were induced. Roots had both tetraploids and octoploids induced by treatment and there were a very few cases where heptoploids were found. Treatment did not induce sterile polyploids such as triploids and pentaploids in roots.

Colchicine treatment induced chimeras (mixture of diploid-polyploid cells) in both leaves and roots. However, roots had some cases where pure polyploids (tetraploids or octoploids) were found. Polyploid chimeras (mixture of induced polyploids) were also induced in roots and they were mainly fertile (tetraploid-octoploid) chimeras.

Regardless of chimeras, colchicine treatment induced dwarfism in both *C. palmata* and *L. sphaerica* species, with significance for plants grown in the beginning of winter (April). Treated plants grown in mid-winter (June) assumed dwarfism when they were young (after two months) but as soon as they grew (after four months) and spring started, they elongated their stems at a faster rate than the control. This elongation made treated plants to be either taller or the same length as their diploid relatives.

The *C. palmata* plants with induced dwarfism had significantly many more stems compared with the control, especially at an age of four months. Treated *L. sphaerica* plants did not have significant stem branching compared to the

control. Treated plants grown in the beginning of winter had significantly reduced leaf area in both species, compared with the control. Mid-winter and summer grown, treated plants, however, had fluctuations in the leaf area compared to the control. There was no significant difference in the number of leaves per plant between treated and control plants in both species.

An increase in plant height caused an increase in shoot fresh and dry weight, and vice-versa. In *L. sphaerica*, the increase in plant height increased the root fresh and dry weight but *C. palmata* treated plants, though they were dwarf, had an increase in root fresh and dry weight compared to the control. Treated plants had low shoot percentage moisture content compared to the control, especially *C. palmata* but *L. sphaerica* had some fluctuations especially for plants grown in mid-winter. Treated roots had significantly higher percentage moisture content compared to the control, for both species, although there were some minor fluctuations.

Treatment induced a significant reduction in the number of stomata in leaves. This was, however, disturbed by the ploidy status of the leaves investigated. Since the results from flow cytometry indicated that there was a high percentage of diploid-polyploid chimeras in leaves including sterile polyploids, some leaves had their replicates indicating diploids and others polyploids. There was also a significant increase in guard cell length for *L. sphaerica* leaves only. In *C. palmata*, guard cell's length is not associated with reduction in the number of stomata but with an enlargement of epidermal cells. Results for this enlargement of the epidermal cells were not recorded. Colchicine treatment resulted in an increased chlorophyll content of the leaves. It also caused the stability of chlorophyll during leaf yellowing. Thus even at that stage, treated leaves had high chlorophyll contents compared to the control.

5.1.2 Conclusion

In conclusion, it is recommended that freshly collected mature *C. palmata* and *L. sphaerica* seeds are used for polyploidy induction since they have a high viability compared to old seeds. Seedlings should be hardened for a short period under low light and then gradually grown in higher light intensities. This especially applies to *L. sphaerica*. Seedlings must be grown in the soil without plant bags or pots to eliminate the pot effect. One day treatment after three days of incubation for germination (3.1DT) is preferred to one day treatment before incubation for germination (1DT). This is because 3.1DT showed high fertile polyploid cells/tissues induction. The 0.01g/L colchicine concentration was shown to be a moderate colchicine concentration for seed treatment. Average number of leaves per plant per treatment criterion seemed to show no significant difference between treated and control plants and thus it is not a good parameter to evaluate the effect of colchicine treatments on plants. In both species, polyploidy can enable a better yield of used parts, such as leaves and roots, in summer, and also the growth of these plants in winter as they are stress tolerant.

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APPENDIX I: Research Questionnaire

Research Questionnaires

Date:
Questionnaire No.
Name of the Interviewer:

Particulars of the area

Name of the Location/Area:
Name of the Sub-location/Sub-Area:
Name of the Village (Precise place):

Sociodemographic data

Name of Respondent (optional):

Gender:

Male	
Female	

Age:

15-24	
25-34	
35-44	
45-54	
55-64	
Over 65	

Plant Species Particulars

Scientific name:
Zulu name:
English name:
Origin of the name:

Source of plant material:

Collected from the wild	
Cultivated	

If cultivated:

When is the plant cultivated (season)?	
Wetland	
Dry land	
Shady places	
Open sunlight	
Irrigation (Yes/No)	
Fertilization (Yes/No)	
Organic or Inorganic Fertilizer: Name	
When is the harvest (season)?	For Food: For Medicine:

Plant usage and Collection

Plant Usage and Collection	**Food**	**Medicine**
What is the use of plant?		
Which part is used?		
Are the plants sold?		
Which plant parts?		
In which state are the plants sold? (Fresh or Dry)		
If collected from the wild, when? (season)		
Any specific time for collection during the day?		
What places does the plant prefer to grow in? (wetland, dry land, grassland, forests, old fields, as weeds among the plants)		

Collecting method:

Preparation Method: Food:

Preparation Method: Medicine:

Storage Method: Plant Material:

Storage Method: Food

Storage Method: Medicine

Dosage

Group of people using the medicine:

Male	
Female	
Both	

Age Group:

Infants	
Children	
Adults	

APPENDIX II: Seed germination and plant growth tables

Table 4.4: Effect of seed treatment before incubation for germination, on percentage germination of *C. palmata* and *L. sphaerica* seeds

Treatment	[Colch.] g/l	*C. palmata*				*L. sphaerica*			
		Day 6		Day 8		Day 6		Day 8	
		Germ. %	SD	Germ. %	SD	Germ. %	SD	Germ. %	SD
0DT	0	40	10	57	6	74	21	94	12
1DT	0.001	10	10	20	17	60	20	67	21
	0.005	24	6	54	6	40	10	50	17
	0.01	14	15	40	20	67	6	77	6
	0.05	17	15	40	0	47	31	67	29
	0.1	7	12	37	23	37	38	44	32
3DT	0.001	20	20	30	30	60	20	67	15
	0.005	17	15	34	12	64	21	74	21
	0.01	20	10	47	6	54	6	64	15
	0.05	14	15	37	6	30	10	44	6
	0.1	20	10	47	6	37	12	77	15
10DT	0.001	24	15	27	15	77	15	77	15
	0.005	30	17	44	15	47	40	57	42
	0.01	27	6	34	6	27	31	27	31
	0.05	27	17	57	12	37	21	44	15
	0.1	24	15	60	0	47	6	74	6

Legend: Germ. %: germination percentage; SD: standard deviation; DT: duration of seed treatment with colchicine in days (day(s) treatment); [Colch.]: colchicine concentration in grams per litre

Table 4.5: Effect of seed treatment after incubation for germination, on percentage germination of *C. palmata* and *L. sphaerica* seeds

Results were recorded at 14 days incubation.

Treatment	[Colch.] g/l	*C. palmata*		*L. sphaerica*	
		% germ.	SD	% germ.	SD
Control	0	60	6	50	8
3.1DT	0.001	37	12	20	6
	0.01	40	8	33	6
	0.1	6	3	17	12
3.3DT	0.001	33	15	27	10
	0.01	40	12	40	8
	0.1	40	10	30	12
6.1DT	0.001	30	10	33	7
	0.01	37	8	37	9
	0.1	40	15	30	12
6.3DT	0.001	40	15	40	12
	0.01	40	9	57	8
	0.1	50	10	47	10

Legend: [Colch.]: colchicine concentration in g/l

Table 4.8: Effect of one day treatment before (1DT) and after three (3.1DT) days Incubation on the germination of *C. palmata* (7 months) and *L. sphaerica* (26 months) seeds

Day	*C.palmata*						*L. sphaerica*					
	Control		1DT		3.1DT		Control		1DT		3.1DT	
	Germ. %	SD	Germ. %	SD	Germ. %	SD	Germ. %	SD	Germ. %	SD	Germ. %	SD
6	32.8	15.91	34.1	18.64	28.1	16.01	9.4	8.34	4.1	6.38	5	5.16
7	34.4	15.59	35	19.92	31.9	17.4	10.9	9.44	5.3	6.7	8.1	6.02
8	36.9	15.8	36.6	19.89	32.8	17.41	14.7	10.87	8.4	10.28	12.8	11.54
9	37.8	15.81	37.8	20.25	34.4	17.5	15.3	12.04	10.3	10.14	15.6	12.63
10	38.8	16.71	37.8	20.25	34.4	17.5	16.9	13.15	12.8	11.69	20	11.69
12	42.2	19.23	40	21.91	37.8	18.25	17.8	13.66	18.1	15.37	23.4	11.65

Legend: Germ.: germination; SD: standard deviation

Table 4.10: Fresh weight changes during germination of *C. palmata* and *L. sphaerica*

Day	C. palmata						L. sphaerica					
	Fresh Wt (g)	SD	% Fresh Wt.	SD	% Germ.	SD	Fresh Wt (g)	SD	% Fresh Wt.	SD	% Germ.	SD
0	0.017	0.0004	0	0	0	0	0.075	0.004	0	0	0	0
1	0.036	0.0016	113.31	13	0	0	0.118	0.008	57.82	3	0	0
2	0.038	0.0019	125.15	13	0	0	0.118	0.007	58.39	4	0	0
3	0.037	0.0023	119.12	14	0	0	0.121	0.007	61.88	4	2	4
4	0.039	0.0012	132.28	10	0	0	0.124	0.009	66.71	8	22	8
5	0.04	0.0019	136.91	10	22	15	0.135	0.012	81.38	12	62	26
6	0.042	0.0023	152.43	13	30	16	0.151	0.016	102.05	19	70	23
7	0.045	0.0043	165.37	23	44	17	0.18	0.022	140.74	28	86	17
8	0.049	0.0074	188.82	40	48	16	0.211	0.037	183.15	46	88	13
9	0.054	0.009	221.03	49	48	16	0.259	0.045	247.2	54	88	13
10	0.058	0.0121	243.24	67	50	17	0.305	0.059	308.35	74	88	13
11	0.067	0.0153	295.59	86	52	19						
12	0.087	0.0368	414.93	213	52	19						

Legend: SD: standard deviation; Wt.: weight in grams; Germ.: germination.

Table 4.11: Fresh and dry weight changed during germination of *C. palmata* and *L. sphaerica* seeds

Species	Day	Initial Wt (g)	SD	Fresh Wt (g)	SD	Dry Wt (g)	SD	% Fresh Wt	SD	% Dry Wt	SD	% Germ	SD
C. palmata	0	0.016	0	0.016	0	0.015	0.001	0	0	-4.17	7.22	0	0
	1	0.017	0.001	0.03	0.003	0.017	0.001	73.15	17	-3.7	6.41	0	0
	2	0.017	0.001	0.039	0.003	0.016	0	123.15	11	-7.41	6.41	0	0
	3	0.019	0.001	0.043	0.003	0.018	0	129.26	24	-3.33	5.77	0	0
	4	0.018	0	0.039	0.001	0.015	0.001	118.52	6	-14.81	6.41	7	12
	5	0.017	0.001	0.037	0.001	0.016	0	120.37	8	-3.7	6.41	7	12
	6	0.017	0.001	0.045	0.002	0.015	0.001	157.87	4	-11.57	0.8	7	12
	7	0.017	0.001	0.043	0.005	0.014	0	155.56	10	-15.74	5.61	47	12
	8	0.018	0	0.05	0	0.015	0.001	177.78	0	-14.81	6.41	47	12
	9	0.017	0.001	0.041	0.003	0.016	0	143.98	6	-3.7	6.41	60	0
	10	0.017	0.001	0.055	0.001	0.015	0.001	233.33	29	-7.87	6.85	67	23
L. sphaerica	0	0.071	0.004	0.071	0.004	0.068	0.003	0	0	-4.64	1.49	0	0
	1	0.075	0.004	0.123	0.006	0.069	0.002	63.92	10	-8.7	5.19	0	0
	2	0.073	0.007	0.121	0.012	0.062	0.005	65.47	4	-15.49	2.16	0	0
	3	0.075	0.005	0.123	0.003	0.067	0.003	64.6	7	-10.63	1.97	0	0
	4	0.069	0.007	0.122	0.008	0.061	0.006	76.4	7	-12.5	0.93	53	12
	5	0.063	0.013	0.107	0.01	0.054	0.012	79.25	21	-14.16	2.82	20	35
	6	0.071	0.004	0.125	0.004	0.061	0.003	77.67	9	-14.1	2.33	27	31
	7	0.073	0.001	0.127	0.031	0.066	0.002	75.62	45	-9.18	1.67	67	42
	8	0.079	0.001	0.167	0.019	0.067	0.002	112.65	23	-14.43	1.67	73	12
	9	0.077	0.006	0.165	0.017	0.068	0.007	116.79	40	-11.43	2.48	60	20
	10	0.075	0.009	0.15	0.012	0.067	0.008	101.14	31	-11.52	0.86	40	35

Legend: SD: standard deviation; Wt.: weight in grams; Germ.: germination.

Table 4.12: Effect of light intensity on plant morphology

Criteria	Light intensity (µmole/m^2/s)	*C. palmata*		*L. sphaerica*	
		Average	SD	Average	SD
Plant height (mm)	<300	274	148.53	746	110.41
	400	202	102.99	234	99.56
	600	177	79.97	202	45.65
	800	53	8.17	124	74.25
	>1000	34	8.91	100	21.71
Stem branching (stems per plant)	<300	1	0	1	0.55
	400	1	0	2	0.84
	600	1	0.45	2	1.3
	800	1	0	1	0.45
	>1000	1	0	1	0.45
Leaf area (cm2)	<300	113	44.58	735	79.55
	400	55	17.64	520	231.44
	600	50	17.41	389	27.25
	800	6	3.15	252	88.67
	>1000	3	0.67	132	38.19
Shoot fresh wt. (g)	<300	1.84	0.71	16.94	2.06
	400	1.73	0.84	10.23	3.53
	600	1.63	0.51	7.77	3.86
	800	0.64	0.16	5.86	2.07
	>1000	0.26	0.11	2.77	1.13
Shoot dry wt. (g)	<300	0.23	0.1	1.76	0.25
	400	0.18	0.1	1.1	0.35
	600	0.23	0.08	0.86	0.46
	800	0.08	0.03	0.7	0.23
	>1000	0.04	0.02	0.32	0.11
Root fresh wt. (g)	<300	1.32	0.74	1.55	0.8
	400	1.43	0.45	1.83	0.85
	600	2.66	1.08	1.63	0.88
	800	0.73	0.84	1.52	0.44
	>1000	0.23	0.14	0.77	0.18
Root dry wt. (g)	<300	0.12	0.06	0.14	0.05
	400	0.14	0.04	0.2	0.07
	600	0.27	0.09	0.18	0.1
	800	0.08	0.09	0.19	0.06
	>1000	0.03	0.01	0.08	0.01

Legend: Wt.: weight; SD: standard deviation

Table 4.15: Ploidy ratio in relation to 2n (where 2n=1) in leaves and roots from one day treated *C. palmata* and *L. sphaerica* seed before and after three and six days incubation for germination

Species	Tt.	Leaves		Fine roots		Bulbous roots	
		4n	8n	4n	8n	4n	8n
C. palmata	Cont.	0.6		1.31		1.8	1.42
	1DT	2.3	0.72	1.27		1.09	
	3.1DT	6.22	2.02	2.68	1.01	3.54	2.86
	6.1DT	0.63	0.62	1.75	0.66	3.06	2.69
L. sphaerica	Cont.	0.59	0.18	1.78	0.97		
	1DT	0.64		2.13	1.04		
	3.1DT	0.88		2.7	1.25		
	6.1DT	0.88	0.16	2.66	1.25		

Legend: Tt.: treatment; Cont.: control; 2n: diploid; 4n: tetraploid; 8n: octoploid

Table 4.19: Effect of polyploidy induction on *C. palmata* and *L. sphaerica* morphology and physiology

Growth period	Plant age (months)	Criteria	Tt.	*C. palmata*		*L. sphaerica*	
				Average	SD	Average	SD
April-February (BW)	3	Plant height (mm)	Cont.	137.66	40	53.33	18
			1DT	131.89	68	27.93	11
			3.1DT	91	66	38.6	29
			6.1DT	98.08	32	43.1	20
		Stem branching (stems per plant)	Cont.	1	0	1	0
			1DT	1	0	1	0
			3.1DT	1	0	1	0
			6.1DT	1	0	1	0
		Foliation (leaves per plant)	Cont.	6	0.45	5.83	0.72
			1DT	6	1	4.67	1.15
			3.1DT	6	1.51	4.4	0.89
			6.1DT	6	0.58	5.25	0.96
		Leaf area (cm^2)	Cont.	50.4	5	109	22
			1DT	37	6	63.4	26
			3.1DT	30.8	6	60.8	14
			6.1DT	39.4	7	64	22
		Shoot fresh wt. (g)	Cont.	5.99	1.13	15.16	4
			1DT	3.28	0.25	6.1	3.16
			3.1DT	2.49	0.88	5.15	2.15
			6.1DT	5.04	2.04	9.68	1.54
		Shoot dry wt. (g)	Cont.	0.67	0.12	1.24	0.11
			1DT	0.48	0.04	0.7	0.32
			3.1DT	0.35	0.11	0.59	0.22
			6.1DT	0.64	0.22	1.07	0.25
		Root fresh wt. (g)	Cont.	0.86	0.26	1.26	0.18
			1DT	0.62	0.14	0.79	0.13
			3.1DT	1.25	0.53	1.03	0.25
			6.1DT	1.07	0.31	0.9	0.2
		Root dry wt. (g)	Cont.	0.17	0.04	0.27	0.08
			1DT	0.13	0.03	0.17	0.03
			3.1DT	0.24	0.11	0.25	0.07
			6.1DT	0.21	0.02	0.18	0.06
		Shoot moisture content (%)	Cont.	88.8	0.61	91.46	2.12
			1DT	85.47	0.73	88.45	0.8
			3.1DT	85.68	1.09	88.5	0.48
			6.1DT	86.96	1.09	89.04	1.16

Legend: Tt.: treatment; [Colch.]: colchicine concentration in g/l; SD: standard deviation; BW: plants grown at the beginning of winter

Table 4.19: Effect of polyploidy induction on *C. palmata* and *L. sphaerica* morphology and physiology (continued)

Growth period	Plant age (months)	Criteria	Tt.	*C. palmata*		*L. sphaerica*	
				Average	SD	Average	SD
April-February (BW)	3	Root moisture content (%)	Cont.	79.68	1.25	78.49	4.37
			1DT	78.44	0.93	78.9	2.63
			3.1DT	80.67	1.05	76.06	2.18
			6.1DT	79.47	5.14	80.07	2.44
		Chlorophyll content (Acetone) (mg/cm^3)	Cont.	6.8	0.2	8.24	0.3
			1DT	7.65	0.3	8.12	0.5
			3.1DT	8.11	0.7	9.79	0.5
			6.1DT	6.86	0.3	10.56	1.2
	4	Plant height (mm)	Cont.	1890.83	499	1669	360
			1DT	1001.79	517	370	221
			3.1DT	122.14	80	178	170
			6.1DT	1478.57	435	1226	459
		Stem branching (stems per plant)	Cont.	1	0.42	1	0
			1DT	2	0.82	1	0.44
			3.1DT	2	1.13	1	0.45
			6.1DT	2	0.97	1	0.32
		Foliation (leaves per plant)	Cont.	19	5.79	12	1.49
			1DT	24	3.73	10	4.08
			3.1DT	7	4	7	2.59
			6.1DT	23	15.76	12	5.67
		Leaf area (cm^2)	Cont.	45.2	1	123.8	9
			1DT	46.2	8	74.2	14
			3.1DT	13.4	5	46.8	12
			6.1DT	51.2	4	97.8	10
		Stomatal frequency	Cont.	23	2.28	49	2.97
			1DT	25	3.77	33	5.54
			3.1DT	23	3.54	20	1.82
			6.1DT	22	0.84	39	6.1
		Shoot fresh wt. (g)	Cont.	20.23	5.47	41.12	27.12
			1DT	23.74	18.81	12.97	4.18
			3.1DT	2.69	0.56	6.95	5.35
			6.1DT	15.73	3.22	33.5	20.15
		Shoot dry wt. (g)	Cont.	2.52	0.56	4.48	2.78
			1DT	2.98	2.12	1.06	0.15
			3.1DT	0.39	0.07	0.86	0.59
			6.1DT	1.71	0.3	3.18	1.97

Legend: Tt.: *treatment*; [Colch.]: colchicine concentration in g/l; SD: standard deviation; BW: plants grown at the beginning of winter

Table 4.19: Effect of polyploidy induction on *C. palmata* and *L. sphaerica* morphology and physiology (continued)

Growth period	Plant age (months)	Criteria	Tt.	*C. palmata*		*L. sphaerica*	
				Average	SD	Average	SD
April-February (BW)	4	Root fresh wt. (g)	Cont.	3.26	1.39	2.19	1.03
			1DT	1.31	0.45	0.63	0.13
			3.1DT	1.32	1.01	0.55	0.28
			6.1DT	3.11	1.56	1.29	0.54
		Root dry wt. (g)	Cont.	0.63	0.46	0.6	0.28
			1DT	0.32	0.12	0.13	0.11
			3.1DT	0.2	0.13	0.24	0.1
			6.1DT	0.34	0.21	0.32	0.11
		Shoot moisture content (%)	Cont.	87.41	0.98	88.96	0.81
			1DT	86.89	1.04	91.47	1.79
			3.1DT	85.56	0.85	86.16	3.15
			6.1DT	89.11	0.36	90.49	0.93
		Root moisture content (%)	Cont.	79.01	13.13	72.65	1.7
			1DT	74.7	7.96	80.52	12.17
			3.1DT	83.37	2.41	53.57	5.63
			6.1DT	89.5	1.34	74.26	7.43
		Chlorophyll content (Acetone) (mg/cm^3)	Cont.	7.35	0.3	11.06	0.5
			1DT	7.31	0.4	12.71	0.6
			3.1DT	8.06	0.3	10.17	0.6
			6.1DT	9.41	0.3	12.21	0.5
	10	Root Fresh wt. (g)	Cont.	14.32	10.79		
			1DT	101.03	20.63		
			3.1DT	42.5	28.7		
			6.1DT	27.72	32.2		
		Root dry wt. (g)	Cont.	1.67	1.92		
			1DT	14.38	3.35		
			3.1DT	4.86	2.5		
			6.1DT	3.91	4.4		
		Root water content (%)	Cont.	88.97	4.35		
			1DT	85.81	0.38		
			3.1DT	87.82	1.99		
			6.1DT	85.05	5.19		

Legend: Tt.: treatment; [Colch.]: colchicine concentration in g/l; SD: standard deviation; BW: plants grown at the beginning of winter

Table 4.19: Effect of polyploidy induction on *C. palmata* and *L. sphaerica* morphology and physiology (continued)

Growth period	Plant age (months)	Criteria	Tt.	*C. palmata*		*L. sphaerica*	
				Average	SD	Average	SD
June – February (MW)	2	Plant height (mm)	Cont.	186	44	125	88
			1DT	126	32	165	88
			3.1DT	155	48	68	37
		Stem branching (stems per plant)	Cont.	1	0	1	0
			1DT	2	0.74	1	0
			3.1DT	3	0.83	1	0.35
		Foliation (leaves per plant)	Cont.	8	1.17	6	1.25
			1DT	10	1.73	7	0.75
			3.1DT	12	3.44	8	2.51
		Shoot fresh wt. (g)	Cont.	7.38	1.34	22.87	6.28
			1DT	9.12	3.09	27.82	5.49
			3.1DT	11.23	3.31	9.59	4.61
		Shoot dry wt. (g)	Cont.	0.76	0.17	2.03	0.58
			1DT	0.87	0.28	2.44	0.5
			3.1DT	1.13	0.29	0.93	0.39
		Root fresh wt. (g)	Cont.	1.12	0.6	1.34	0.16
			1DT	1.01	0.37	1.5	0.41
			3.1DT	1.15	0.33	0.93	0.16
		Root dry wt. (g)	Cont.	0.3	0.21	0.37	0.08
			1DT	0.32	0.07	0.38	0.1
			3.1DT	0.31	0.11	0.22	0.06
		Shoot moisture content (%)	Cont.	89.79	0.43	91.16	0.11
			1DT	90.39	0.28	91.25	0.2
			3.1DT	89.85	0.43	90.08	0.7
		Root moisture content (%)	Cont.	74.54	8.51	72.21	2.81
			1DT	66.82	6.31	74.51	1.19
			3.1DT	73.54	2.74	76.72	3.71
		Chlorophyll content (Acetone) (mg/cm^3)	Cont.	10.5	0.3	10.46	0.4
			1DT	9.14	0.2	11.09	0.5
			3.1DT	11.36	0.2	9.89	0.4
	3	Chlorophyll content (DMSO) (mg/cm^3)	Cont.	30	2	31	2
			1DT	17	2	32	4
			3.1DT	23	3	28	4

Legend: Tt.: treatment; [Colch.]: colchicine concentration in g/l; SD: standard deviation; MW: plants grown in mid-winter

Table 4.19: Effect of polyploidy induction on *C. palmata* and *L. sphaerica* morphology and physiology (continued)

Growth period	Plant age (months)	Criteria	Tt.	*C. palmata*		*L. sphaerica*	
				Average	SD	Average	SD
June – February (MW)	4	Plant height (mm)	Cont.	2300	1224	1632	1363
			1DT	1979	1177	2677	986
			3.1DT	2117	1489	1780	1376
		Stem branching (stems per plant)	Cont.	3	1	4	1.15
			1DT	4	0.58	2	0.58
			3.1DT	4	0.58	5	0
		Foliation (leaves per plant)	Cont.	77	62	59	14
			1DT	72	22	54	11
			3.1DT	54	36	63	8
		Leaf area (cm^2)	Cont.	63.2	9	132.4	22
			1DT	46.4	6	151	49
			3.1DT	84.8	12	123.2	18
		Shoot fresh wt. (g)	Cont.	37.74	19.24	101.67	46.79
			1DT	39.27	21.63	106.48	38.54
			3.1DT	36.08	23.69	135.58	77.92
		Shoot dry wt. (g)	Cont.	4.29	2.2	13.1	5.64
			1DT	6.11	4.34	16.25	5.51
			3.1DT	5.92	4.24	15.47	9.51
		Root fresh wt. (g)	Cont.	5.48	2.15	3.4	1.02
			1DT	33.49	37.53	4.96	1.36
			3.1DT	10.3	7.79	3.26	2.66
		Root dry wt. (g)	Cont.	0.7	0.17	1.15	0.32
			1DT	3.24	3.14	1.44	0.62
			3.1DT	1.81	1.19	1.14	1.12
		Shoot moisture content (%)	Cont.	88.61	0.7	86.93	1.52
			1DT	85.2	3.39	84.66	0.75
			3.1DT	83.99	1.51	88.75	0.55
		Root moisture content (%)	Cont.	86.42	3.48	65.91	2.11
			1DT	88.89	1.93	71.56	4.57
			3.1DT	79.21	7.09	68.91	9.13
		Chlorophyll content (Acetone) (mg/cm^3)	Cont.	9.502	0.7	13.054	0.5
			1DT	9.736	0.3	17.528	0.7
			3.1DT	12.696	0.2	17.07	0.7

Legend: Tt.: treatment; [Colch.]: colchicine concentration in g/l; SD: standard deviation; MW: plants grown in mid-winter

Table 4.19: Effect of polyploidy induction on *C. palmata* and *L. sphaerica* morphology and physiology (continued)

Growth period	Plant age (months)	Criteria	Tt.	*C. palmata*		*L. sphaerica*	
				Average	SD	Average	SD
June – February (MW)	5	Chlorophyll content (DMSO) (mg/cm^3)	Cont.	15	3	26	3
			1DT	19	4	24	3
			3.1DT	22	4	29	5
	8	Root Fresh wt. (g)	Cont.	6.32	6.3		
			1DT	24.18	17.4		
			3.1DT	40.71	24.9		
		Root dry wt. (g)	Cont.	0.84	0.8		
			1DT	2.45	1.6		
			3.1DT	4.5	3.5		
		Root moisture content (%)	Cont.	84.93	7.53		
			1DT	88.57	4.21		
			3.1DT	89.01	2.86		
January – January (S)	3	Leaf area (cm^2)	Cont.	51.4	12		
			1DT	39.1	7		
			3.1DT	35.9	4		
		Chlorophyll content (Acetone) (mg/cm^3)	Cont.	6.33	0.5	12.25	0.5
			1DT	6.95	0.4	12.99	0.1
			3.1DT	7.44	0.2	11.52	0.4
	12	Chlorophyll content (Acetone) (mg/cm^3)	Cont.	9.12	3	11.99	1
			1DT	11.97	1	11.02	2
			3.1DT	12.84	2	12.82	2

Legend: Tt.: treatment; [Colch.]: colchicine concentration in g/l; SD: standard deviation; MW: plants grown in mid-winter, S: summer