

**OPTIMIZATION OF BULBLET PRODUCTION BY LEAF  
CUTTINGS IN *LACHENALIA*.**

**BY**

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# OPTIMIZATION OF BULBLET PRODUCTION BY LEAF CUTTINGS IN *LACHENALIA*.

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## ABSTRACT

*Lachenalia* is a genus endemic to South Africa and Namibia. It is propagated vegetatively by daughter bulbs, bulbils, tissue culture and leaf cuttings. In Europe, the demand for *Lachenalia* bulbs is estimated at 20 million per annum and thus the need for a rapid and cheap way of propagation. Of all the vegetative methods for propagating *Lachenalia*, the easiest and cheapest method is by leaf cuttings. Cook first reported this method of propagation in 1931. Nothing was done at that time, as the natural way of propagation sufficed for the demand. No efforts were, however, made to optimize the technique of leaf cuttings.

In this study various aspects aimed at optimizing the production of bulblets by leaf cuttings of *Lachenalia* were studied. These included: stage of the donor (mother) plant, leaf section position, medium, disinfectants, and starch deposition

in leaves and bulbs during the growing season. Other aspects studied included the ontogenetic origin of bulblets and roots on leaf cutting.

The optimal physiological stage to take leaf cuttings was found to be when the inflorescence was visible between the sheath of the leaves and the worst stage was at full flowering. As the donor plant matures, there was a reduction in the number, size and mass of bulblets produced by leaf cuttings. The proximal sections performed better than their distal counterparts in all variables evaluated.

Considering cost and time, applying disinfectants when planting the leaf cuttings seem not to be necessary. Decomposed bark was the best medium for *Lachenalia* leaf cuttings.

Bulblets were mostly formed on the adaxial leaf surface on *Lachenalia* leaf cuttings and developed as a result of the division of the epidermal cells. Both the leaf cutting and the bulblet formed roots. On the leaf cuttings, roots originated from the parenchyma cells associated with the vascular system while on the bulblets they developed from the base of the meristematic mass of cells, which formed the bulblets, and were attached to the bulblet. In a TEM study of the leaf, no starch was observed in leaf sections. Starch was, however, observed in all sections from bulb parts. More starch grains were observed on the inflorescence stalk than in other bulb parts.



## List of Abbreviations

- df – degree of freedom  
SS – sum of squares  
MS – mean square  
MSE – mean square error  
FV – F-value  
LSD - least significant difference  
% - percent  
NS – not significant  
C.V. – coefficient of variance  
CV – cultivar (s)  
P - position  
H – healing  
D- disinfectant  
S - stage  
F - fertilization  
M - medium  
°C - degrees centigrade  
cm - centimeter  
g - gram  
KNO<sub>3</sub> - potassium nitrate  
CaNO<sub>3</sub> - calcium nitrate  
l - Litre  
mm – millimeter  
S- river sand  
DB- decomposed pine bark  
ARC-Roodeplaat – Agricultural Research Council, Roodeplaat Vegetables and Ornamental Plant Institute

## CHAPTER 1

### INTRODUCTION

#### 1.1 The genus *Lachenalia*.

*Lachenalia* Jacq.f. ex Murray is a genus consisting of approximately 110 species (Duncan, 1988). They are bulbous geophytes belonging to the family Hyacinthaceae (formerly Liliaceae) and are endemic to South Africa and Namibia (Duncan, 1988; Perrignon, 1992). *Lachenalia* mostly grows in the Southern and Southwestern Cape and Namaqualand (Duncan, 1988).

The bulb of *Lachenalia* is tunicate, having an outer, soft or hard dry membranous covering which protects it from drying out and physical injury (Duncan, 1988). The fleshy bulb consists of inner and outer bulb-scales, and a disc-like, rudimentary stem. The outer bulb-scales function as storage organs and contain reserve food material while inner bulb-scales (leaf bases) protect the central growing shoot, which produces leaves and flowers annually. The shape and size of bulbs vary from species to species. One, two or more leaves are formed and the length, number and shape of leaves vary according to species.

Three different forms of inflorescences are encountered in the genus. Firstly the spike, where the flowers are sessile and attached directly to the rachis; secondly



the subspicate inflorescence, where the flowers are attached to the rachis by very short pedicels and thirdly the raceme, where the flowers are attached to the



Figure 1.1: *Lachenalia* cultivars; Namakwa, Robyn and Romelia.

rachis by long pedicels (Duncan, 1988). *Lachenalias* are characterized by tubular or bell shaped flowers ranging from shades of red, green, blue, purple, yellow and white (Duncan, 1988; Hancke and Liebenberg, 1990). Some flowers are brightly colored (Figure 1.1)

## 1.2 Economic importance.

*Lachenalia* cultivars can be used as cut flowers, potted bulbs, and garden bulbs (Niederwieser *et al*, 1997). *Lachenalia* cultivars are multiplied vegetatively to obtain true to type planting material. The potential market for selling *Lachenalia* pot plants in Europe is estimated at twenty million bulbs per annum (Niederwieser *et al*; 1997), hence the need for a rapid and cheap way of propagation.

The local *Lachenalia* pot plant market is very small as compared to the export market. As the production of bulbs is in South Africa, and due to the amount of technical work needed on *Lachenalia* growing, it also creates job opportunities for unemployed South Africans. Foreign capital is earned by exporting dry bulbs to Europe where the bulbs are grown and sold as pot plants (Figure 1.2).

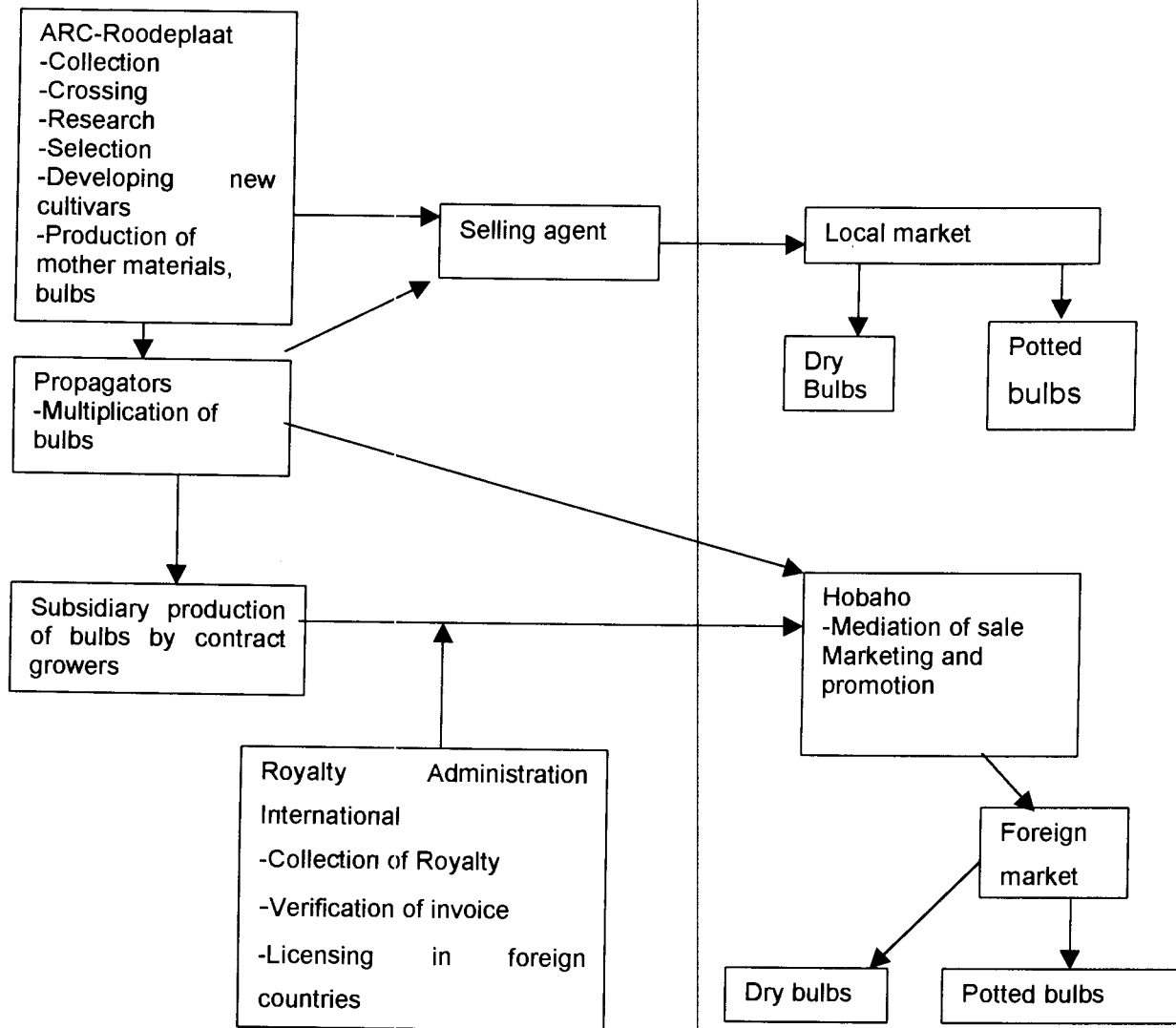


Figure 1.2: Flow diagram of *Lachenalia* cultivar development as done at ARC-Roodeplaat.

### 1.3 Vegetative propagation of *Lachenalia*.

Some *Lachenalia* cultivars are sterile but most are fertile (Riana Kleynhans, pers. Comm.). *Lachenalia* cultivars are propagated vegetatively because of variations that exist when seeds are used and thus, true to type materials cannot be obtained. The variation that exists is interesting, as at ARC-Roodeplaat, there are two different cultivars, which originated from the same parents.

When seeds are used for propagation, plants take three years to come to flower where-as if leaf cuttings are used; they flower a year or two sooner. A number of species reproduce readily by forming daughter bulbs.

*Lachenalia* can be propagated vegetatively in a number of ways such as offsets, bulbils, leaf-cuttings and tissue culture (Duncan, 1988; Klessner and Nel, 1976; Niederwieser and Vcelar, 1990; Perrignon, 1992; Suh et al, 1997). According to Duncan (1988), offsets (natural) are side bulbs which develop inside the mother bulb and are separated during the dormant period whereas bulbils are bulblets which form naturally on the adaxial surface of leaf bases, and at the end of short stolons which develop at the base of the bulb.

As far as leaf cuttings are concerned, it has been reported that any *Lachenalia* leaves which were cut or broken off and left on moist gravel did not shrivel, but remained in a fresh condition for a considerable time (Cook, 1931). On closer

examination it was noticed that the leaves were beginning to heal over the point of severance, not by ordinary callus, but by means of a corky covering and bulblets were formed (Cook, 1931). Nothing was done at that time to use leaves as cuttings for propagation because the stocks of bulbs were sufficiently large enough for the requirements. As for now, the demand for bulbs had increased and hence the need to optimize the leaf cutting method of propagation.

According to Naylor (1940), cuttings from the flower stalk of both *Lachenalia tricolor* Thunb. and *Hyacinthus orientalis* L. Getrude, develop bulblets after four weeks in moist sand. Tissue culture is used for the multiplication of new hybrid cultivars at ARC-Roodeplaas. It is also a means of producing virus free materials, which in *Lachenalia*, is an important consideration (Duncan, 1988).

#### 1.4 Problem statement.

The *Lachenalia* international pot plant market is hampered by lack of a sufficient quantity of bulbs and thus the need for a rapid means of propagation. *Lachenalia* can be propagated by offsets, but the negative aspect about offsets is that only one to four offsets can be formed on one mother bulb for most cultivars. The same applies to bulbils. The major problem with these two methods is that not all varieties have the potential of forming daughter bulbs. Tissue culture is a rapid way of propagating *Lachenalia* although the disadvantage is that it is more expensive than leaf cuttings. Propagation of *Lachenalia* by leaf cuttings is a

cheaper method of multiplication than tissue culture and can also be applicable to small-scale farmers and for rural community projects. More information about refinement of this method is however, required.

#### 1.5 Research objectives.

The main objective of this research investigation was to optimize the production of *Lachenalia* bulblets by leaf cuttings. In addition, the origin of bulblets on leaf cutting was determined by means of light and electron microscopy.



## CHAPTER 2

# LITERATURE REVIEW: VEGETATIVE PROPAGATION OF BULBOUS PLANTS.

### 2.1 Introduction.

It is important to understand the meaning of vegetative propagation and bulbous plants before the two can be dealt with together. Vegetative propagation is the use of a source (mother) plant to produce plants identical in genotype (cloning). New roots and/or shoots are regenerated on stems, leaves, or roots (cuttings and layers) (Hartmann *et al*, 1990). Bulbous plants include those that produce bulbs, corms or tubers.

According to Rockwell and Grayson (1953), bulbs in reality, are buds, in a state of dormancy waiting to resume growth, only at the right season or proper conditions of temperature and moisture. Doerflinger (1973) describes a bulb as a bud sheathed in layers of food-storing fleshy white leaves called scales attached to a tough flat disc or basal plate (compact stem) and from this base emerge both the roots and the stem of the flower or inflorescence.

A corm has an enlarged stem (basal plate) that has distinct nodes and internodes (De Hertogh and Le Nard 1993). Tubers are composed of solid tissue, but do not have a basal plate (Rockwell and Grayson, 1953). It is a modified stem, which

have discernible 'eyes', or buds that develop into new growth shoots e.g. potatoes (Rockwell and Grayson, 1953). Since *Lachenalia* has a true bulb, the rest of this study will be confined to bulbs.

To multiply a particular variety of bulb rapidly, it is often necessary to use artificial techniques because under natural conditions, the rate of natural multiplication is generally slow. Bulbs can be propagated vegetatively by offsets, bulbils, division, scooping, scales, leaf-cuttings, tissue culture, chipping, cross-cutting, bulblets and scoring (Rockwell and Grayson, 1953; Doerflinger, 1973; Browse, 1979; Duncan, 1988; Van der Linde, 1992; Van Leeuwen and van der Weijden, 1997; Mori *et al*, 1997; Sander-Ziv *et al*, 1997).

## 2.2 Vegetative methods of propagating bulbous plants.

### a) Leaf-cuttings.

When propagating by leaf cuttings, the leaf blade is utilized in initiating new plants. Adventitious buds and adventitious roots form at the base of the leaf and develop into a new plant (Hartmann *et al*, 1990). Cutting across prominent veins can help to induce plantlet formation on the wound (Rees, 1972).

Detached easter lily leaves will form bulblets at the leaf base when put on wet medium (Miller, 1992b). The detached leaf cuttings should be buried 1.5cm in moist vermiculite at 70°F. The leaf cutting form an average of about two bulblets per leaf. Leaves from the upper portion of the plant provide the best success (80%) regeneration, and basal leaves the lowest (40%) (Miller, 1992b).

Cuttings from leaves such as Cape cowslips (*Lachenalia*), snow drops (*Galanthus*) and snowflakes (*Leucojum*) tend to wilt quickly, so they should be kept turgid by planting as soon as possible (Browse, 1979). Leaf-cuttings from bulbous plants such as *Hyacinthus*, that have tender leaves may well rot and die unless they are handled as little as possible, planted carefully and sprayed carefully with fungicide (Browse, 1979).

Cook (1931) first reported the production of bulblets *in vivo* from leaves of *Lachenalia*. Since then, propagation of *Lachenalia* by this method has been referred to by numerous authors (Mahlstede and Haber, 1957; Crosby, 1986; Duncan, 1988; Perrignon, 1992; Suh *et al*, 1996). Whole leaf cuttings perform better than their half-length counterparts but if the maximum number of bulblets is desired, leaves should be cut into lengths of eight or nine centimeter (Duncan, 1988; Perrignon, 1992). Tissue obtained from the proximal end of the leaf produce more bulblets than those from the distal end, both *in vitro* (Niederwieser and Vcelar, 1990) and *in vivo* (Perrignon, 1992).

b) Daughter bulbs.

Daughter bulbs (referred to as offsets in other literature) are side bulbs that develop inside the mother bulb and are separated from the mother bulb during the dormant period (Duncan, 1988). According to Doerflinger (1973) and Witham Fogg (1974), offsets are those bulbs that are formed at or near the base of the parent bulb. These bulbs are big enough to flower the following year e.g. *Narcissus* bulbs grow bigger year by year, it fragments into two to four smaller bulbs of sufficient size to flower the following year. With tulips and certain other bulbs, the mother-bulb disappears, but not before producing two to three bulbs capable of flowering the following year.

Rockwell and Grayson (1953) reported that an offset starts as a bud and then gradually increases in size until it breaks away from the mother bulb and becomes independent from the mother bulb. The advantage of propagating bulbs through offsets is that true to type planting material is obtained. Not all *Lachenalia* species reproduce readily by this method (Duncan, 1988). In those species where daughter bulbs do appear, they appear in limited numbers, too small for rapid propagation. Bulblets are tiny bulbs that develop below the ground on some bulbs (Browse, 1979). In late summer, bulblets should be removed gently; the bulb and bulblets should be planted straight into the ground at twice their own depth. Few species naturally produce daughter bulbs in any quantity,

some important species, such as *Lilium auratum* and *L. tigrinum*, produce a small number.

c) Bulbils.

Certain *Lachenalia* species produce small bulbs referred to as bulbils, at or above ground level in leaf axils. An example of this is *L. bulbifera* of which some produce bulbils along the entire adaxial margin of the leaf-base. These bulbils may be removed at the end of the growing season and stored until autumn (Duncan, 1988).

According to Browse (1979), bulbils are tiny bulbs that grow in the leaf axils on above ground stems of certain species of lily such as *Lilium tigrinum*. After flowering time, the bulbils are collected off the plant as they mature. They are set 2.5 cm apart in a pot filled with compost or similar medium, covered with grit and placed in a cold frame. In autumn of the following year, they are transplanted into the ground (Browse, 1979). Lilies produce aerial bulbils, which are perfectly formed, pea-sized miniature bulbs in leaf axils. They fall away from the mother plant soon after flowering (Doerfinger, 1973 and Fox, 1985). These bulbils, under favourable condition begin to grow and become bulbs capable of flowering in a year or two. Miller (1992b) reported that many species of *Lilium* produce structures that are outgrowths of axillary buds associated with the leaves of the upright shoot. These are aerial bulbils and are usually dark in colour; and are

comprised of several shortened scales and a meristem. Roots are usually not seen on bulbils (Miller, 1992b). When these structures are removed and placed in moist substrate, roots are formed, followed by leaf and shoot development. Usually two to three years of growth is necessary for flowering to occur depending on species and cultivar (Miller, 1992b).

A number of lily species such as *Lilium candidum* can be artificially induced to produce bulbils by disbudding the plant just before flowering (Browse, 1979). Bulbils will develop in the leaf axils during the remainder of the growing season and they are collected as they mature. They are planted in compost or a similar medium. They are left for at least twelve months, until autumn of the following year and are transplanted to open ground (Browse, 1979). Certain other plants can also be artificially induced to produce bulbils (Browse, 1979; Rix, 1983 and Bryan, 1989). The problem is that they are small and they normally flower, two years after their formation (Duncan, 1988).

#### d) Chipping.

Chipping is a technique where a bulb is cut longitudinally into a number of segments (chips) of equal size; by this action the main growing point is destroyed and the apical dominance is broken. In *Chionodoxa*, *Galanthus*, *Muscari* and *Scilla*, the highest yield (number of bulbs and total weight) was obtained by

chipping as soon as possible after lifting (in spring) (Van Leeuwen and Van der Weijden, 1997). The highest yield was always obtained by planting the chips immediately after chipping (van Leeuwen and van der Weijden, 1997).

In *Eucomis comosa*, the highest yield was obtained by chipping in summer and twelve-week storage at 17° to 23°C (Van Leeuwen and Van der Weijden, 1997). Lifting the bulbs a year after chipping results in loss of very small bulblets and this lead to the decrease in propagation rate. "Outer" half-chips gave higher yield than the "inner" halves (16.2 and 12.3 respectively) (Sandler-Ziv *et al*, 1997).

#### e) Scoring.

Flower bulbs can be produced in a short time by using a technique called scoring which makes fewer, larger bulblets as there are less cut scale leaf surfaces as in hyacinth (Browse, 1979). If the bulb is large, four scores can be made; if the bulb is small, two scores at right angles will be enough (Browse, 1979). The scored bulbs should be stored in a warm (21°C) dry environment for a day and this will cause the cuts to open out (Browse, 1979). The cut surface should be dusted with a fungicide. Bulblets produced in this way usually require a further two to three years to reach flowering size (Browse, 1979). This technique can be used on many bulbous plants like hyacinth, *Lachenalia*, *Muscari*, *Narcissus*, *Scilla*, Snowdrop (*Galanthus*), Snowflake (*Leucojum*) etc. (Browse, 1979).

f) Scooping.

Scooping is a technique that is halfway between cutting and scaling (Rockwell and Grayson, 1953). Commercial growers employ it in propagating garden hyacinths as this increases the number of bulblets as compared to offsets (Rockwell and Grayson, 1953). Browse (1979) wrote that scooping is carried out towards the end of the bulb's dormant period.

Browse (1979) reported that to scoop the bulb successfully, and with minimum damage, requires a special tool such as an old teaspoon with one sharpened edge. This teaspoon should be used to cut out the basal plate in one scooping movement, leaving the rest of the bulb undisturbed and the cut surface of all scales exposed (Rockwell and Grayson, 1953; Browse, 1979).

Although it is possible to do this by knife, it is inadvisable as the center of the bulb may become macerated and subject to rotting (Browse, 1979). Once the basal plate has been removed, the cut scale leaf surfaces should be dusted with a fungicidal powder to minimize microbial attack. The bulb should be placed upside down, with the scale leaf bases exposed, on a wire tray or a tray containing dry sand (Browse, 1979).

The scooped bulbs should be placed at a temperature of at least 21°C to encourage callus formation on the scale leaf bases and so further combat any



chance of infection; and the scooped bulbs should be kept as dry as possible, but ensure that the scale leaves do not desiccate (Browse, 1979). An airing cupboard is probably a suitable environment, but dampen the sand occasionally. In about two to three months, new bulblets will develop on the cut surfaces of the scale leaves (Browse, 1979). The bulbs are then planted in pots, and should be placed upside down so that the bulblets are just below the surface of the compost.

In spring, the bulblets will grow and produce leaves and the old bulb will gradually disintegrate (Browse, 1979). At the end of the season the bulblets should be lifted, separated, and replanted. Normally they can take three to four years before the flowering size is reached (Browse, 1979). This technique can be used on many bulbous plants like hyacinth, *Lachenalia*, *Muscari*, *Narcissus*, *Scilla*, Snowdrop (*Galanthus*), Snowflake (*Leucojum*) etc. (Browse, 1979).

g) Stem-layering.

Stem layering is an easier and quicker method than scaling in lilies, but it produces fewer new plants (Rockwell and Grayson, 1953). Bulblets develop in greater numbers than they would normally form, or where they could not form at all under normal conditions. In lilies stem layering is accomplished by removing the stem from the bulb; just as the flower wither, by giving it a quick pull or jerk with a twisting motion (Rockwell and Grayson, 1979). Stems are gathered and

heeled in, to about one third their length, in well-drained sandy soil or a half-and-half mixture, by bulk, of sand and peatmoss. Placing the stems on a slant will permit covering them with sash in an ordinary cold frame (an advantage with late-flowering cultivars), as they can be held over in the frame until spring.

A modification of this method is to cut the stem back to 30.48cm or 60.96cm, all leaves are removed, layered in sand or sand-peatmoss, covered a couple of centimeters deep, in a frame until the bulblets have been formed (Rockwell and Grayson, 1953).

#### h) Crosscutting.

Bulbs that are slow to multiply naturally may be divided by cutting across the base of the bulb. This method has been widely used in commercial production of hyacinths and amaryllis (Mori *et al*, 1997). The bulb is left in one piece but the cuts go through the basal plate and the apical meristem into the scales. The bulb should then be kept in a warm place such as indoors in a polythene bag with barely moist peat while the bulblet form. If possible, the bulbs should be stood upside down so that the bulblets grow straight. When the bulblets are formed, the whole bulb should be planted again upside down, and grown for a year. By the end of this, the young bulbs are large enough to detach and be grown on. When the mother bulbs of *Nerine sarniensis* were placed directly on the medium without curing after cross cutting, bulblets formation on rice-chaff charcoal

medium was better than that on vermiculite, sand or perlite medium (Mori *et al*, 1997). The curing treatment at 25°C for two weeks appeared to be appropriate as there was 100% bulblet formation, and larger bulbs were obtained. Bulblet formation was not affected by the time of crosscutting. The results of Mori *et al* (1997) show that larger mother bulbs produce larger bulblets following incubation for about six months.

i) Bulblet formation on flower stems.

Plants that produce bulblets like hyacinth can be artificially induced to increase their bulblet production. This is done by removing the flower stem, and burying it until bulblets develop in the leaf axils (Browse, 1979). The stem should be sprayed with a liquid fungicide to prevent diseases. A trench should be dug 15cm deep, slope one side up to ground level. The trench should be filled with sand or light compost. By autumn, bulblets will have developed in the leaf axils at the lower end of the stem (Browse, 1979). These can be detached and planted straight into the ground at twice their own depth or be left *in situ* for a year. This is an easy method of producing bulblets, and the only difficulty likely to arise is the microbial attack on the stem before the bulblets are produced (Browse, 1979; Bryan, 1989).

Miller (1992b) reported that stem bulblets in lilies are expanded buds from axils of underground leaf nodes. The stem bulblets are complete and consist of roots,

scales, basal plate, and meristem. Bulbs are dug and the detached stem bulblets are replanted in the field in August or September (Miller, 1992b). The principal advantage of using stem bulblets relative to scaling is the saving of full year production time and that the clone is maintained (Miller, 1992b).

j) Scaling.

Some of the true bulbs can be propagated rapidly by an operation known as scaling. Lilies are usually grown in this way (Rockwell and Grayson, 1953). The thick fleshy scales are removed from the outside of the bulb; care should be taken not to bruise them and to retain the base of each. If the apical bud of the bulb is left intact, it can be replanted for further growth. The scales are then set in rows in flat sand or a sand-peatmoss mixture, and covered about 5 centimeters deep (Rockwell and Grayson, 1953). In hyacinth, a technique called double scaling is used (Rees, 1972). They should be kept covered against rain, and only moderately moist, for twenty to twenty-five days, until miniature new bulbs begin to form at the bases of the old scales; then moisture can be increased. Temperature of about 60°F should be maintained (Rockwell and Grayson, 1953).

The best time for scaling lilies and for cutting bulbs is when their food reserves are at a maximum, during their dormant stage before new root growth has started (Rix, 1983). Adventitious buds are formed during the winter season, and develop into small bulbs or 'scalets'. The scalets sprout in spring and form small plants

(Miller, 1992b). The adventitious scalets are mostly associated with a wounded surface (Miller, 1992b).

It is possible to increase the number of bulblets by cutting into the basal section of a broad scale with a razor-sharp blade, e.g. a scale with a base 2.5cm long may be given three cuts 5mm deep; four bulbs may then be formed instead of one or two (Fox, 1985). Bulbs with scales, such as lilies and fritillaries, have relatively small, narrow scale leaves which can readily be pulled off the basal plate of the bulbs (Browse, 1979). Scales should be taken from a fresh bulb in a turgid condition by pressing the scale leaves outwards until they snap off close to the basal plate of the bulb.

Any scale leaves will carry rotting agents and so they must be protected by using a fungicidal powder. The scale should be placed in a polythene bag filled with fungicidal powder such as Captan™.

In early spring the bulblets will produce leaves above the compost. In summer, the plants harden off. At the end of the season, when leaves have died down, the young bulbs can be lifted and separated (Witham Fogg, 1974; Browse, 1979; Rix, 1983; Bryan, 1989).

k) Division.

This technique starts with an operation in which some section of parent plant is removed and this piece is then induced to form roots and becomes a new plant (Rockwell and Grayson, 1953). In making divisions it is essential that there be a growing point (a bud or 'eye') on each piece.

Browse (1979) reported that bulbs propagate naturally by division. In annual growth cycle the apical bud develops and produces a new bulb during the growing season. If an axillary bud develops into an active growing point, and then this also develops as a bulb that may take a year or two of further growth before it separates from its original parent and eventually starts flowering. In some plants, notably tulips and bulbous irises, the original bulb disintegrates after flowering, leaving a cluster of small bulbs as well as a new flowering bulb. They should be pulled apart in autumn and planted out at twice their new diameter (Browse, 1979).

l) Tissue culture.

Tissue culture has evolved as a powerful tool for bulb breeders and propagators during the last decades. This technique has been developed for the production of plants from various tissues. According to Van der Linde (1992), tissue culture is the culture of plants or plant parts on an artificial medium in a controlled and

sterile environment. Plants may originate from explants, meristems, gametes, protoplasts, fused cells or transformed cells, thereby providing the basis for the development of methods to accelerate clonal propagation (Van der Linde, 1992). The interesting aspect of tissue culture is that every part of the plant may be used: root, leaf, stem, flowerbud, microspore, bulb etc. The medium may vary between sterilized water in glass pearls and highly nutritious composition in an agar-based gel (Van der Linde, 1992).

In *Lachenalia*, tissue culture propagation of cultivars is necessary as plants are susceptible to virus infection which causes the leaves and the inflorescence to be commercially unacceptable (Niederwieser, 1990).

Lilies are easy to establish in tissue culture, with generally favourable propagation rates (Miller, 1992b). Agar culture is the basic method in lily tissue culture propagation. Scales are surface sterilized for twenty minutes in 10% clorox solution, with a detergent. After rinsing with sterile water, scales are sectioned and placed onto the medium. New structures invariably arise on the basal portion of the scale, and also on the inner side of the scale (Miller, 1992b; Bryan, 1989).

### 2.3 Why using leaf cuttings in *Lachenalia*.

The advantage of this method of propagation is that many bulblets can be formed on one leaf cutting and that a number of leaf cuttings can be obtained from one donor plant (Perrignon, 1992; Suh *et al*, 1997). Duncan (1988) reported that it is an effective method of increasing stock of species, mainly those that do not set seeds readily like hybrids. Other reasons are that it is commercial viable, inexpensive and that no special tools like and cabinets needed when propagating by leaf cuttings.

### 2.4 The terms bulblet, bulbil and offset.

The cited authors referred to in literature review used the terms bulblets, bulbils and offsets in an attempt to link the name to the origin of bulblet for example, a small bulb or bulblet derived from either an axillary bud or adventitious buds on a specific position on the plant or plant organ. Since it is not always possible to undoubtedly identify the position of origin, it is difficult to differentiate between them. For this reason, in the rest of the thesis, only one term will be used, namely bulblet(s).



## CHAPTER 3

### ORIGIN OF BULBLETS AND ROOTS ON *LACHENALIA* LEAF-CUTTING PLANTED *IN VIVO*.

#### 3.1 Introduction.

*Lachenalia* leaf cuttings form bulblets and roots when severed and placed in the soil or growing medium (Cook, 1931; Duncan, 1988; Naylor, 1940; Perrignon, 1992; Suh *et al*, 1997). In herbaceous plants adventitious roots usually develop just outside and between the vascular bundle cells but tissues involved at the site of origin depend on the kind of plant (Hartmann *et al*, 1997). In *Lachenalia* leaf cuttings, it is not clear as to exactly from which tissue in the leaf the new structures (bulblets and roots) form.

The aim of this chapter was to observe the origin of bulblets and roots on *Lachenalia* leaf cuttings planted *in vivo*.

#### 3.2 Materials and Methods.

##### General view on *Lachenalia* leaves.

The mature bulb of most *Lachenalia* species produce two leaves while in other species, a single leaf is produced. There is a remarkable variation within the genus and several species have numerous grass-like leaves. Leaves vary from robust and broad to short and cylindrical in shape. Many

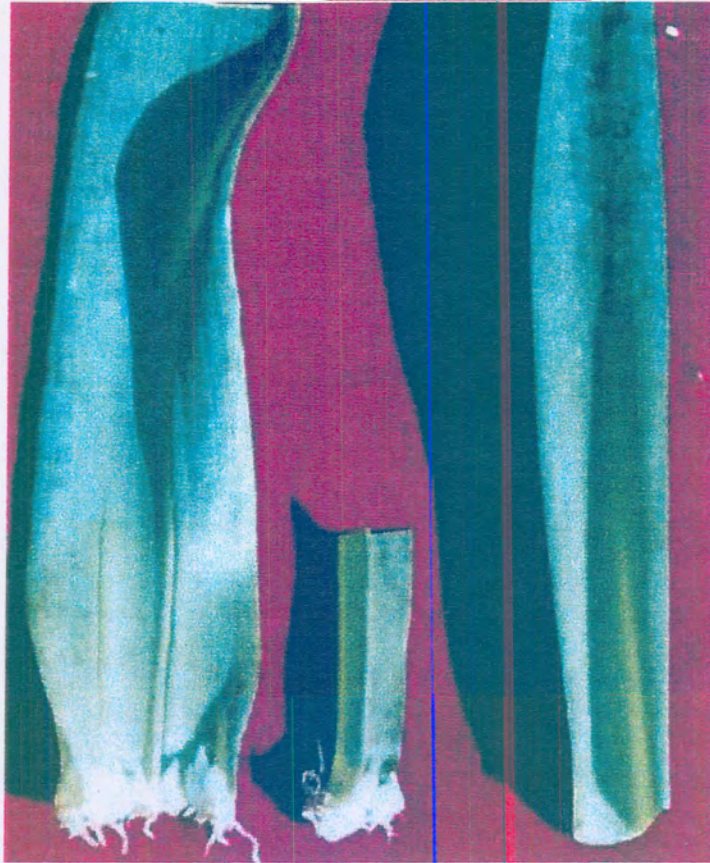


Figure 3.1: *Lachenalia* leaf cuttings showing root development and bulblet formation.

species have conspicuous features on leaves like spots and banding. The colour and density of spots varies with locality and temperature e.g. *L. rubida*, growing in full sun, usually has conspicuous purplish spots, while those growing in shade have spots in shades of green (Duncan, 1988). Cultivars used in this trial have broad leaves.

Mature bulbs of a hybrid B27 (an ARC-Roodeplaat hybrid) were planted in pots and placed in a glasshouse maintained at 20°C and 10°C, day and night

respectively. The medium that was used for the mother bulbs was decomposed pine bark. Two bulbs with a five to six centimeter in circumference were planted per pot. Twelve pots were used in this trial and a total of 24 bulbs were planted.

Outer leaves were severed randomly when the inflorescence bud was visible between the leaf sheaths. Leaf cuttings were planted on decomposed bark in crates and were also placed in the glasshouse. The glasshouse temperature was maintained at 25°C and 15°C, day and night respectively. Leaf cuttings were irrigated every third day. For inspection they were uprooted from the medium every day from the second day of planting until the 28<sup>th</sup> day when the bulblets were clearly visible.

Portions of the basal area of the leaf (part where bulblets and roots form) were fixed in gluteraldehyde (GA). After fixing all the samples from the second day of planting to the 28<sup>th</sup> day, the samples were put in fresh GA for one night in the refrigerator. They were then washed with phosphate buffer for 3X15 minutes. Samples were then dehydrated with 50%, 70% and 90% ethanol for 15 minutes in each and then put in 100% Ethanol for 3X15 minutes. Resin impregnation was done by putting the samples in 1:1 solution of 100% ethanol and resin for three hours and finally samples 100% resin. The resin that was used for this imbedding was LR White (a medium grade Acrylic Resin, 5ppm hydroquinone). Samples were then imbedded in capsules with fresh resin. Polymerization was done in an oven. Sections (2-

5mm) were then cut, mounted on slides and stained with toluidine blue. They were put on hot plate to dry out and were ready for light microscope investigation.

### **3.3 Observations and discussion.**

#### **3.3.1 Leaf structure.**

Leaves are amphistomatal (stomata on both the adaxial and abaxial epidermis). The mesophyll consists of polygonal cells, smaller adjacent to the epidermis and larger in the central zone. No palisade cells are present and all mesophyll cells contain chloroplasts. Epidermis cells are much smaller than the mesophyll cells (Figure 3.2)

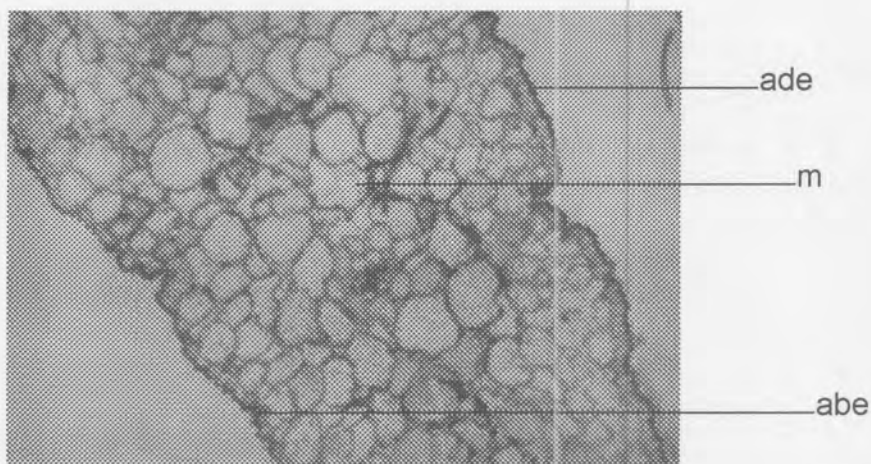


Figure 3.2: Section of basal part of *Lachenalia* leaf (Hybrid B27).

abe- abaxial epidermis

ade- adaxial epidermis

m- mesophyll

### 3.3.2 Origin of new structures.

#### Bulblets.

Bulblets on *Lachenalia* leaf cuttings were observed approximately four weeks after planting. They appeared on both the adaxial and abaxial surface of the leaf cutting, and also several centimeters from the severed or wounded area. However, most bulblets were formed on the adaxial surface of the leaf cutting. Bulblets develop as a result of the division of the epidermal cells (Figure 3.3). Sub-epidermal cells also divide as the bulblet developed.

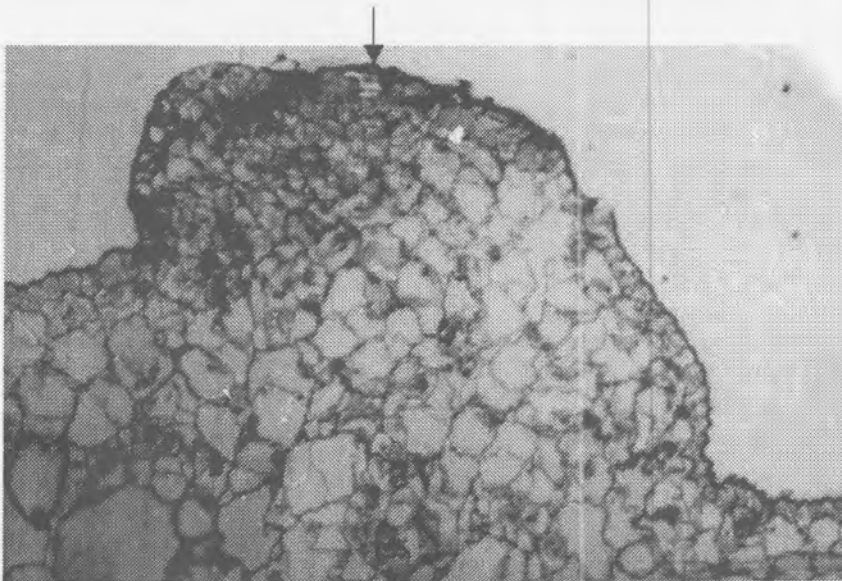


Figure 3.3: Transverse section of leaf cutting base showing the meristematic activity forming the bud primordia that will form the bulb (see arrow).

The observation that bulblets were irregularly distributed along the basal end of the leaf cutting corresponds with the findings of Hussey (1976) that bulbils in *Ornithogalum thyrsoides* originated from the divisions in the epidermis and hypodermis. In onions, adventitious shoots initiated from the epidermis and hypodermis on the abaxial surface of leaves grown *in vitro* (Hussey and Falavigna, 1980). In *Hyacinthus orientalis* (L.) Getrude bulblets originated from the epidermal cells (Naylor, 1940). Walker (1940) found that bulblets originated from the parenchyma cells near the adaxial surface in detached scale leaves of *Lilium candidum* and *L. longiflorum*.

The findings of this investigation contradicted the findings of Suh *et al* (1997) that new bulblets originated from the parenchyma cells near the vascular bundle sheath. According to the results of this investigation, the structures that originated from the parenchyma cells associated with the vascular system were the roots (see page 34). However the results of this investigation that bulblets originated from the epidermis confirmed the findings of Niederwieser (1990) for *Lachenalia* explants *in vitro*.

### 3.3.3 Sprouting of bulblets.

In most cultivars investigated bulblets that were formed on the leaf cuttings did sprout and produced grass-like leaves that supported the small bulblets when the original leaf cutting withered and died. This happened approximately three months after planting the cuttings. Cultivars that sprouted

include Romelia, Ronina, Rupert and Namakwa but Romelia sprouted most readily. Cultivar Robyn did not sprout but produced bigger bulblets than Romelia (Chapter 4).

For readily sprouting cultivars like Romelia, it is important to stop irrigation after the original leaf cuttings had died down so that young grass-like leaves could die down. However, stopping irrigation shouldn't be done too soon, as those grass-like leaves have to support the bulblets. Stopping irrigation too soon might lead to smaller bulblets. There is a perception that for bigger bulblets, irrigation should not be stopped to force the grass-like leaves to die down. However, this perception has to be proved. For this experiment, irrigation was stopped when the original leaf cutting died down so that bulblets could be harvested at the same time. It might be possible that if irrigation was not stopped, bigger bulblets might have been harvested as the grass-like leaves were still actively growing. What seem to happen is that the bulblets get smaller when they sprout, but when the grass-like leaves die down, it seems as if the nutrients are translocated back to the bulblets.

Little is known about factors influencing the senescence of the original leaf cutting and the grass-like leaves that are formed on bulblets. It is evident that high temperatures and water shortage hastens senescence. Even mother bulbs can be forced to die down by putting them at high temperatures and

withholding irrigation. Sprouting seems to be influenced by temperature and water. However this perception needs further investigation.

#### 3.3.4 Roots.

Both the leaf cuttings and the newly formed bulblets formed roots. Roots on leaf cuttings are formed first and those on the bulblets were formed later when the bulblets were growing in size.

Roots on leaf cutting.

Roots on the leaf cuttings developed about four weeks after planting, after bulblets had already developed. They were initiated deep inside the leaf tissue adjacent to the vascular bundle and protruded through the wounded or severed area at the base of the leaf cutting (Figure 3.4).

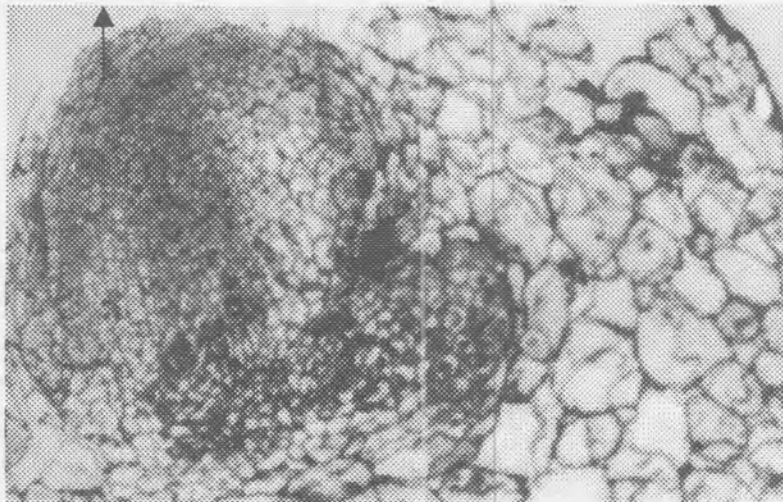


Figure 3.4: Root developing on *Lachenalia* leaf cutting. Arrow shows the direction of growth.



The results support the general concept of endogenous origin of roots and that roots originated from the cells near the vascular system as in *Hyacinthus orientalis* (Naylor, 1940), *Lilium candidum* and *L. longiflorum* (Walker, 1940) and *Ornithogalum thyrsoides* (Hussey, 1976). It should be noted that roots that were formed on *Lachenalia* leaf cuttings as described above, were not associated with the exogenous bulblets.

Further information about adventitious roots formation in other crops are: in sweet potato, where new roots and shoots on leaf cuttings arise in callus tissue, which develops over the cut surface through the activity of secondary meristems (Hartmann *et al*, 1997); in tomato, pumpkin and mung bean, adventitious roots arise in the phloem parenchyma; in *Crassula* they arise in the epidermis where as in *Coleus* they originate from the pericycle (Hartmann *et al*, 1997); in castor bean roots arise from the vascular bundle while in carnation they arise from a layer of parenchyma cells inside a fibre sheath (Hartmann *et al*, 1997).

Roots on bulblets.

As the bulblets grew in size, they formed roots. These roots developed from the base of the meristematic mass of cells that formed the bulblets, at the point of attachment between the bulblet and the leaf cutting. These roots supported bulblets after senescence of the original leaf (Figure 3.5). They

also supported the grass-like leaves in those cultivars that have the characteristic of sprouting.

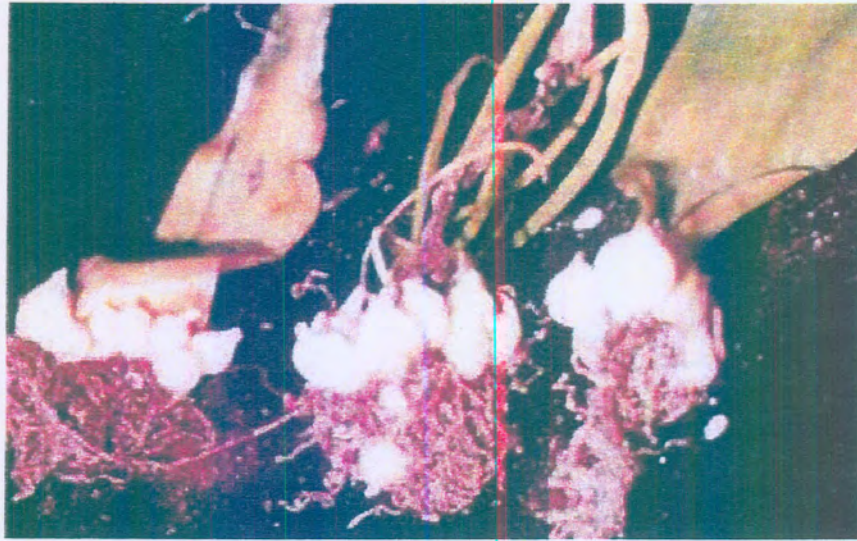


Figure 3.5: *Lachenalia* leaf cuttings showing the roots on bulblets when the original leaf cutting senesce.

### 3.4 CONCLUSION.

Bulblets are mostly formed on the adaxial leaf surface on *Lachenalia* leaf cutting. They develop as a result of the division of the epidermal and sub epidermal cells. Roots on leaf cuttings originated from parenchyma cells associated with the vascular system and they appear externally through the wounded area at the base of the leaf cutting. Roots on bulblets develop from the base of the meristematic mass of cells, which form the bulblets, and are attached to the bulblets.

## **CHAPTER 4**

# **EFFECT OF LEAF-SECTION POSITION AND PHYSIOLOGICAL STAGE OF THE DONOR PLANT ON *LACHENALIA* LEAF-CUTTING PERFORMANCE.**

### **4.1 INTRODUCTION**

The bulblets are formed at the base of the leaf-cuttings after at least four weeks from planting (Chapter 3). From the literature, it is not clear as to when the leaves should be severed from the donor plants. The effect of leaf section position together with the physiological stage of the donor plant on the performance leaf cuttings planted *in vivo* has not yet been studied.

The aim of this trial was to investigate how the physiological stage of the donor plants would affect leaf cuttings performance.

### **4.2 MATERIALS AND METHODS.**

Mature bulbs of three commercial cultivars, 'Romelia', 'Robyn' and 'Rupert' were planted in February in a glasshouse. The temperature in the glasshouse was maintained at 25°C and 15°C, day and night respectively. Decomposed pine bark was the medium used for both donor plants and leaf-cuttings. One tea spoon (5ml) of Fongarid™ (A wettable powder fungicide for the control of

root and stem rot and damping-off caused by *Pythium* and *Phytophthora* in citrus and ornamental nurseries), 60g of dolomite lime and 60g of Osmocote® (six month slow nutrient releasing fertilizer) was added to about 20 litres medium in each crate.

Plastic crates were used to plant the mother plant bulbs as well as the cuttings. One replication was planted per crate. The size of the crate used in this trial was 54cm (length) X 32cm (breath) X 12cm (height). Bulbs with a four to five centimeter in diameter were used. Each cultivar was replicated five times, planting 80 bulbs per crate. A total of 1200 bulbs for all three cultivars were planted.

A total of three hundred cuttings per cultivar per stage were planted; sixty cuttings per crate and thus five replicates. Severed leaves were planted in the same condition as that of the donor plants.

#### Treatments.

To assess the effect of leaf section position on consequent cutting performance, leaves were severed randomly and cut into the proximal and distal parts (cuttings). The distal and proximal cuttings were planted in separate crates.

To determine the effect of physiological stage of the donor plants on the performance of leaf-cuttings, leaves were severed at three different stages (Figure 3.1), namely:

- When the inflorescence bud was visible between the sheath of the leaves,
- Before the first flower opened, and
- At full flowering (when all flowers were open on the inflorescence).

Irrigation was stopped approximately five months after planting the cuttings. Bulblets were harvested after the original leaf cutting had died down, that was approximately three weeks after stopping the irrigation. Bulblets were cleaned by hand and roots were removed prior to evaluation. A fan was used to blow away soil and other foreign materials from the bulblets.

Variables examined were:

- Survival ability of leaf cutting. After eight weeks from planting the number of surviving cuttings were counted and the data recorded.
- Number (quantity) of bulblets produced. After the original leaf cutting died down, bulblets were harvested, cleaned and counted. This was about five months after planting the cuttings.
- Quality. The size of bulblets in circumference and fresh mass of bulblets were measured after counting.

Data collected was subjected to analysis of variance and means were tested via the confident interval of 95% probability. The statistical programme that was used for analyzing the data was SAS.

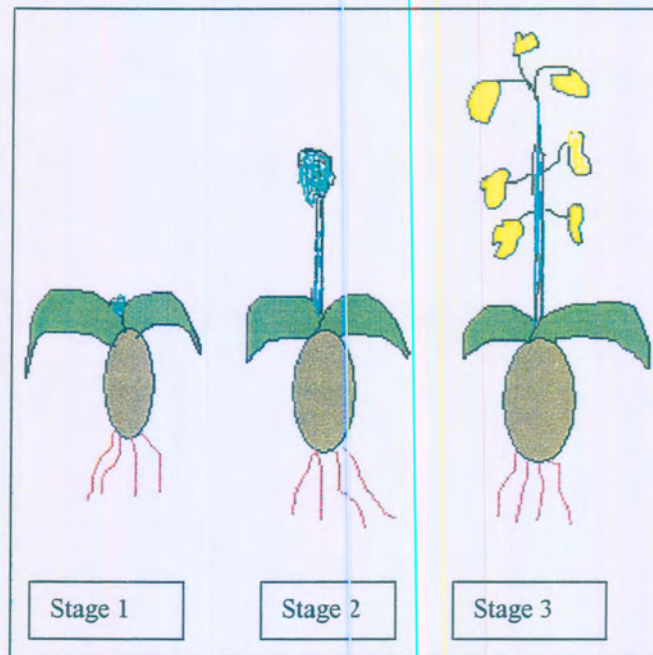


Figure 3.1: *Lachenalia* plants showing the three physiological stages.

Stage 1: Inflorescence visible between sheath of leaves

Stage 2: Before the first floret open

Stage 3: Full flowering

Stage 1 will be referred too as early and stage 3 as late in the text.

### 4.3 Results and discussion.

#### Survival ability.

Both proximal and distal cuttings were viable (surviving) eight weeks after planting. Proximal cuttings had significantly higher survival rate than distal cuttings (Figure 4.1). As the physiological stages of the donor plant progressed, there was a reduction in the number of surviving cuttings (both proximal and distal). Proximal cuttings were not affected to the same degree by physiological stages, compared to their distal counterparts. Survival ability of distal cuttings of cultivar Robyn was low in all three physiological stages tested (Figure 4.1). There was a significant difference between the three physiological stages tested in this trial (Table 1A, appendix).

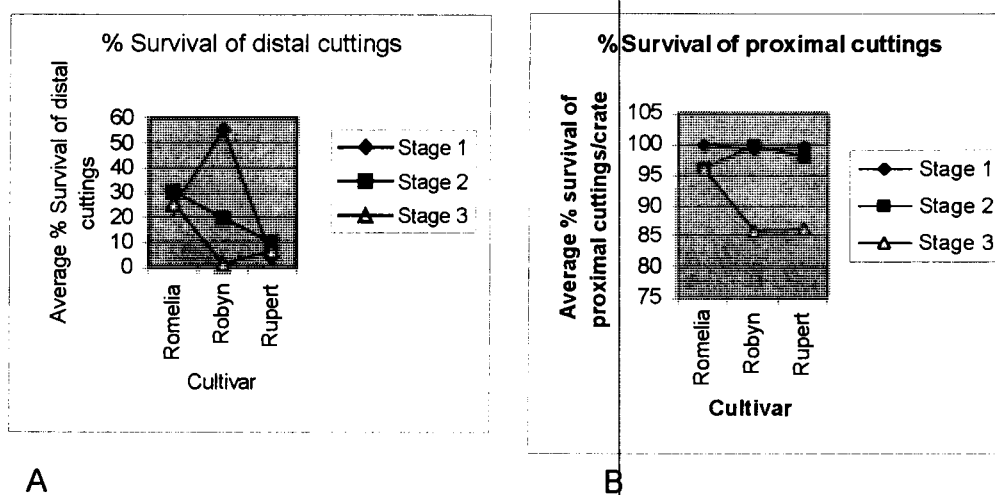


Figure 4.1: Effect of cultivar, position and physiological stage on leaf cutting survival ability.

A: Survival rate of distal cuttings of three *Lachenalia* cultivars.

B: Survival rate of proximal cuttings of three *Lachenalia* cultivars.

### Number of bulblets.

Both the proximal and distal cuttings produced bulblets in all three physiological stages tested but the proximal leaf cuttings produced significantly more bulblets per crate than distal cuttings. Compared to other cultivars, Romelia was far more productive in terms of the number of bulblets (Figure 4.2). Number of bulblets followed the trend of number of cuttings that survived. Distal cuttings of cultivar Robyn almost yielded nothing due to low survival rate of cuttings taken at full flowering. Irrespective of leaf cutting position, cuttings taken when the inflorescence was visible between the sheath of leaves produced the highest number of bulblets (Figure 4.2).

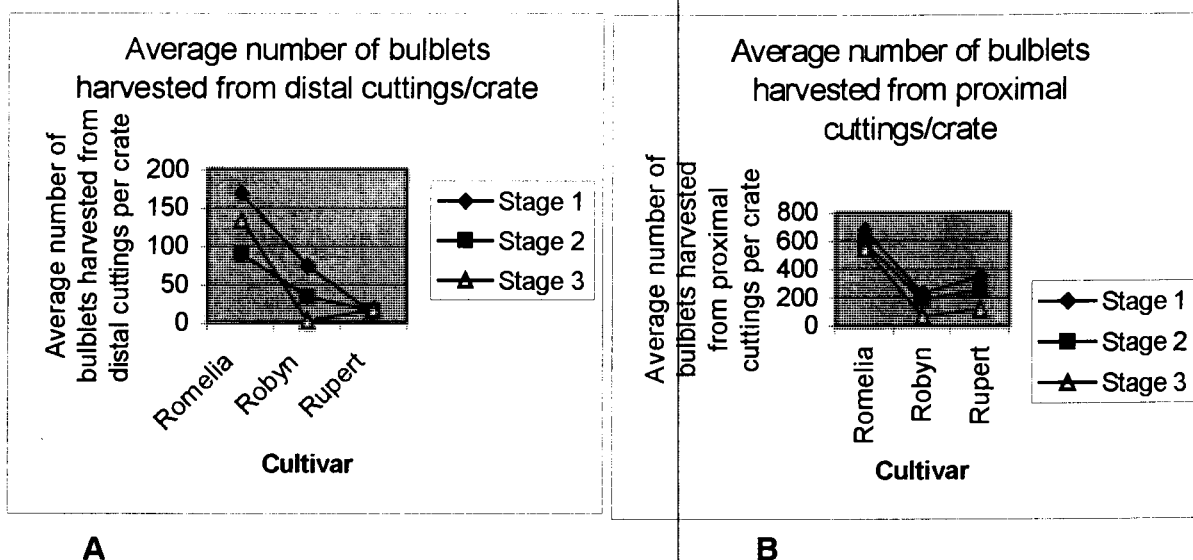


Figure 4.2: Effect of cultivar, position and physiological stage of leaf cuttings on the number of bulblets per crate. A: Distal cuttings of three *Lachenalia* cultivars. B: Proximal cuttings of three *Lachenalia* cultivars.



There was a significant difference between the leaf cutting positions tested in this trial in the number of bulblets produced (Table 1B, appendix). The reason for higher productivity of the proximal section might be because tissues on the distal leaf cuttings are more mature as compared to the young tissue found at the basal (proximal) part of the leaf (Fahn, 1982).

### Mass of bulblets.

Mass of the bulblets per crate followed the same trend as the number of bulblets produced per crate in this investigation since a higher mass was obtained from cuttings severed when the inflorescence was visible between leaf sheaths.

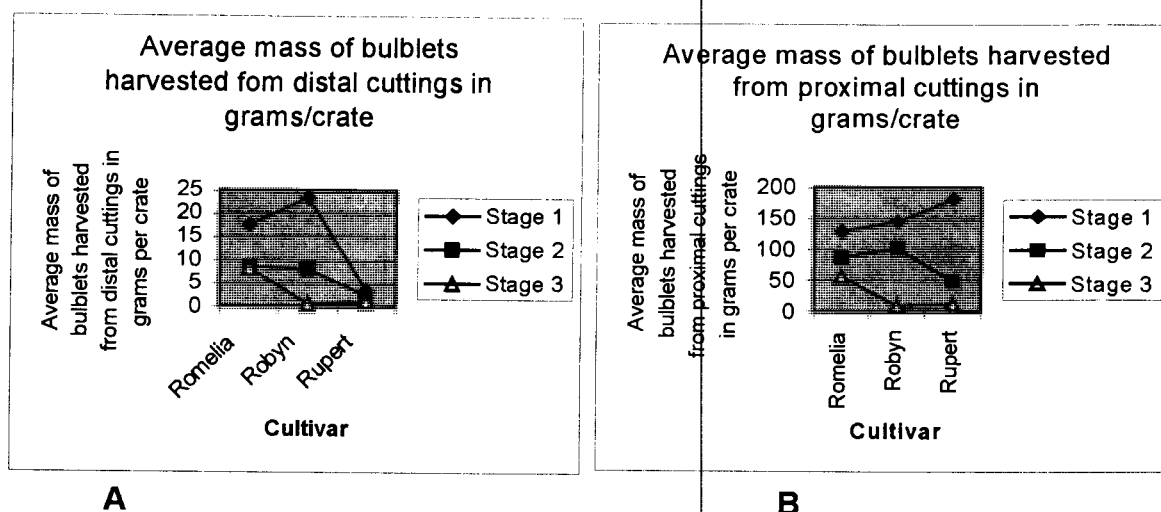


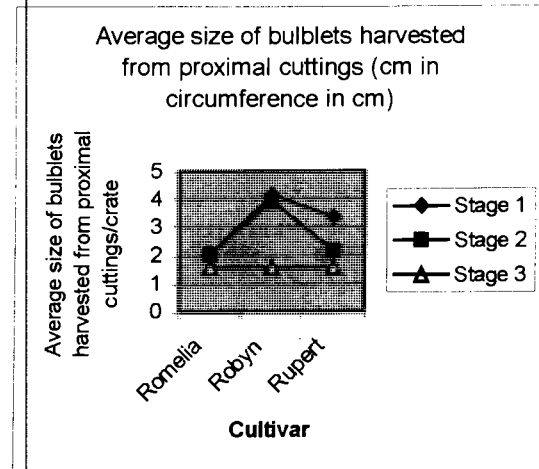
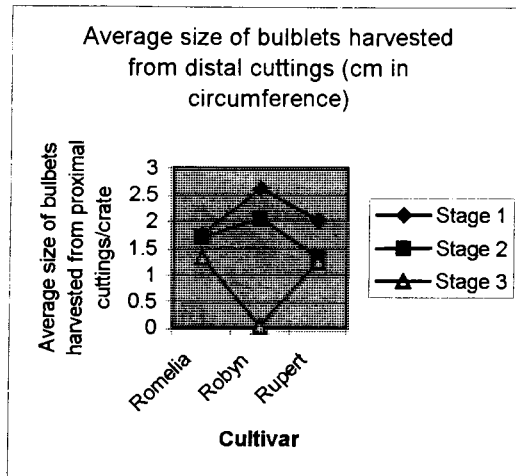
Figure 4.3: Effect of cultivar, position and physiological stage of cuttings on the mass of bulblets produced per crate. A: Mass of bulblets harvested from distal cuttings of three *Lachenalia* cultivars; B: Mass of bulblets harvested from proximal cuttings of three *Lachenalia* cultivars.

Bulblets harvested from proximal cuttings had a significantly higher mass than their distal counterparts (Figure 4.3). There was a significant variation in the mass of bulblets harvested from the two (proximal and distal) leaf-cutting positions (Table 1C, appendix). As in survival rate and the number of bulblets per crate, there was also a reduction in mass of bulblets as the physiological stages of the donor plant progressed. There was no significance variation on the mass between cultivars (Table 1C, appendix).

### **Size of the bulblets.**

Proximal leaf cuttings produced significantly bigger bulblets than distal cuttings. Cultivar Robyn produced bigger bulblets than the other two cultivars, however this difference was not due to the effect of physiological stages, but to cultivar difference although the distal cuttings of Robyn was severely affected by stage 3.

Bigger bulblets were harvested from cuttings severed when the inflorescence was visible between the sheath of leaves and the size decreased as the physiological stages progressed. It is important to take leaf cuttings early, when the inflorescence is visible between the sheath of leaves if bigger bulblets are required.



A

B

Figure 4.4: Effect of cultivar, position and physiological stage of leaf cutting on bulblet size. A: Bulblets harvested from distal and B: proximal cuttings.

'Romelia' seems to be the least affected by the stage when cutting were taken; though for best results, sections should be taken when the inflorescence is visible between the sheath of leaves. When propagating *Lachenalia* by leaf cuttings, there is a relation between regeneration ability, number of bulblets produced, mass and size of bulblets produced.

The results of this trial support the findings of Perrignon (1992) and Suh *et al* (1996) that leaf cuttings obtained from the distal end of the leaf produce less bulblets as compared to proximal part (Figure 4.2). The findings by Niederwieser

and Vcelar (1990), that physiological stage of the donor plant plays an important role in determining the regeneration potential of cultured *Lachenalia* leaf tissue *in vitro* was also confirmed by this trial.

The importance of physiological stage and position of leaf cuttings or leaf explants has often been stressed by authors. In this investigation, leaf cuttings taken earlier (when the inflorescence was visible between the sheath of leaves) gave the best results. It is, however, obvious that the most important aspect is not necessarily the physiological stage, but the age of leaf tissue at the time when cuttings are taken. Considering the development of monocotyledonous leaf (Fahn, 1982); the apical portion is the most mature part of the leaf. Leaf elongation is caused by an intercallary meristem situated towards the distal part of the leaf. During stage 1, when the inflorescence is situated in the leaf sheath, the intercallary meristem is still active, producing young leaf tissue. During stage 3, the meristem is not active anymore, and all the tissues have matured, but the proximal part still contains the youngest tissues. The above reasoning could explain why proximal (basal) leaf cuttings perform better than their distal counterparts in all variables examined.

#### **4.4 CONCLUSIONS.**

For higher productivity of *Lachenalia* leaf-cuttings, it is recommended that leaf cuttings be severed from the base of the leaves when the inflorescence bud is

visible between the sheath of the leaves (Stage 1). The productivity of the proximal sections exceeded that of distal sections for all variables (survival rate, fresh mass, number and size of bulblets) examined. Considering time, space and cost, it is not advisable to take distal cuttings at full flowering (Stage 3).

Distal sections should be taken at an earlier stage for good results. Even though proximal cuttings had higher survival ability than distal cuttings in all three physiological stages tested, proximal cuttings still perform reasonably taken when the inflorescence bud is visible between the sheath of the. Irrespective of the physiological stage when leaf cuttings are taken, cuttings senesce at the same time. In other words, the earlier one takes the leaf-section, the better.

It should be taken into consideration that as the donor plant matures, there was a reduction in the survival rate of the cuttings and number, size and mass of bulblets produced. It is apparently not the physiological stage as such that determines the above parameters, but rather the age of the tissues in the leaf cuttings.

It can therefore be recommended that leaf cuttings containing immature intercalary leaf tissues be used for *Lachenalia* propagation.

## CHAPTER 5

# EFFECT OF GROWING MEDIUM ON *LACHENALIA* LEAF-CUTTING PERFORMANCE.

### 5.1 Introduction.

One of the most important factors in container cultivation is drainage of the growing medium, which must be excellent at all times. Despite the fact that *Lachenalia* in the wild, occur in a wide range of soil types with differing pH values, they generally adapt very easily to other soil types under cultivation (Duncan, 1988). The latter author reported that the main component of the growing medium is sand, which should preferably be medium grained, washed river sand. According to experiences at ARC-Roodeplaat, leaf-cuttings planted in river sand (S) produce fewer but bigger bulblets as compared to the cuttings planted in decomposed bark (DB), which produce smaller, but greater number of bulblets. Another reason why these two media were chosen for this study is that they are readily available. The combination of these two media has not yet been tested for *Lachenalia* leaf-cuttings.

The aim of this chapter was to observe the effect of sand (S), decomposed bark (DB) and different combinations of sand and decomposed bark on the performance of *Lachenalia* leaf-cuttings.

## 5.2 Materials and methods.

Mature bulbs of cultivar Robyn were planted in commercial decomposed bark under a shade structure (55% irradiance) in March. All chemicals that were added were the same as in Chapter 4. Leaf-cuttings were severed in June when the inflorescence was visible between the sheath of leaves and were planted in the glasshouse maintained at 25° and 15°C, day and night respectively.

The number of cuttings planted per crate was 60 and a total of 300 cuttings per treatment were planted, since each treatment was replicated five times. Only the proximal sections were used in this trial because it has been found that they perform better than their distal counterparts (Chapter 4). Cuttings were irrigated three times a week. Osmocote (60g), and 5ml (teaspoon) of Fongarid<sup>R</sup> was added in each crate. The experiment was repeated in the following year.

To assess the effect of different media on *Lachenalia* leaf-cutting performance, the following media were used:

- river sand (S),
- decomposed bark (DB),
- river sand (S) : decomposed bark (DB) (1:1),
- River sand (S): decomposed bark (DB) (3:1).

Criteria that were evaluated were:

- number of bulblets produced,
- diameter of bulblets,
- mass of bulblets.

Bulblets were harvested and weighed after cuttings had died down. Data collected was subjected to analysis of variance and means were tested via the confident interval of 95% probability.

### **5.3 Results and discussion.**

Decomposed bark (DB) produced the highest quantity of bulblets as compared to sand (S) and the combinations of the two media in both two seasons (Figure 5.1). Mass of bulblets produced followed the trend of quantity of the bulblets. This is evident as bulblets harvested from decomposed bark gave the highest mass (Figure 5.1). Fewer and cleaner bulblets were harvested from sand as compared to the bulblets harvested in other media tested in this trial. The quantity of bulblets produced by different media differed significantly (Table 4i and ii, appendix).

Bigger bulblets were harvested from sand in the first season even though the difference with decomposed bark and the combination of the two media (1:1) was



not significant. In the second season, smaller bulblets were harvested from sand though the cuttings were planted on the same day for all media (Figure 5.1). The difference between the two seasons might be because the mother bulbs sprouted and were planted earlier than in the first season. This has led to leaf cuttings being taken earlier and thus had (leaf cuttings) longer time in the medium than in the first season.

Most cuttings died down on sand due to wilting while in decomposed bark were rotting due to higher water holding capacity of the bark. As leaf cuttings planted in sand showed signs of wilting during the first weeks of planting while cuttings planted on decomposed bark retained their vigour, it can be suggested to apply more water or irrigate more frequently in sand than decomposed bark.

Bulblet and root formation started earlier in decomposed bark than sand. It was however surprising as bulblets harvested from sand were slightly bigger than the bulblets harvested from decomposed bark in the first season.

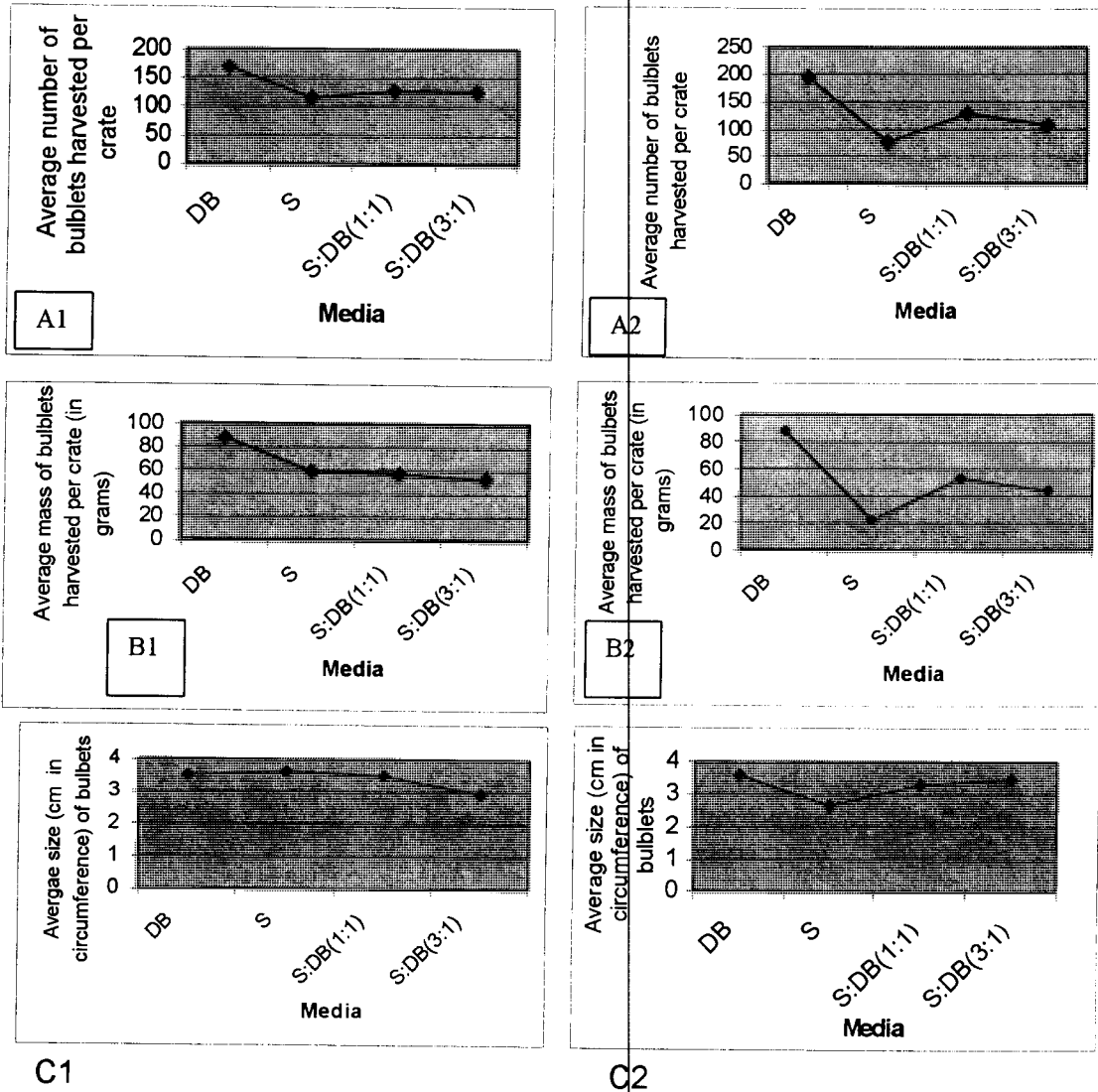


Figure 5.1: Effect of different media on the performance of leaf cuttings.

A: Average number of bulblets produced per crate in season 1 (A1) and season 2 (A2)

B: Average mass of bulblets produced per crate in season 1 (B1) and season 2 (B2)

C: Average size of bulblets produced in season 1 (C1) and season 2 (C2).

Suh *et al.*, (1996) found that the best results for *Lachenalia* leaf cuttings were obtained when the medium composed of peatmoss: perlite (1:1) was used. Even though good results were obtained from the combination of these two media, peat is very expensive. A cheaper medium that is readily available like decomposed bark would be more appropriate.

#### **5.4 Conclusion**

The results obtained in this investigation suggested that decomposed bark can be used for *Lachenalia* leaf cuttings but if fewer, cleaner and bigger bulblets are needed, sand could be used. If sand is used, it should be understood that the frequency of irrigation would be frequent compared to that of decomposed bark.

Considering the costs and labour for mixing the media, the combination of sand and decomposed bark is not recommended as the results of this investigation revealed that pure decomposed bark gave better results in both seasons.

## CHAPTER 6

# EFFECT OF SUPPLEMENTARY FERTILIZATION OF THE DONOR PLANTS ON LEAF-CUTTINGS PERFORMANCE IN *LACHENALIA*.

### 6.1 Introduction.

The fact that hyacinths can be brought to full bloom in pure water only, is a good illustration that all the required nutrients are contained in the bulbs during the dormant period to complete the production of perfect flowers and foliage (BULLETIN no 197, 1967; Duncan, 1988). The latter bulletin again emphasized that fertilizers and manure should never be added to the medium. Extremely acid soils should not be used. Fertilization of *Lachenalia* donor plants before leaf cuttings are taken has not yet been studied. The objective of this Chapter was therefore to study the effect of supplemented fertilization of mother bulbs on leaf cutting performance.

### 6.2 Materials and methods.

Two cultivars, 'Robyn' and 'Ronina'; were examined in this experiment. Glasshouse condition, medium, bulb size and number of bulbs planted per crate were similar to that of assessing the effect of different stages of the plant on leaf-cutting performance (Chapter 4).

Three treatments in the first season were applied in this experiment, namely: calcium nitrate ( $\text{CaNO}_3$ ), Nitrosol and the control (where no additional nutrients

were applied). In the second season a fourth treatment,  $\text{KNO}_3$  was also included. The applied calcium nitrate contained 19.5% of calcium and 15.5% nitrogen and was applied in two crates at rate of 5g/litre. Another two crates were sprayed with potassium nitrate, which contained low chlorine, total nitrogen of 13,5% and 46% potassium oxide soluble in water.

A leaf application of Nitrosol contained 8% nitrogen, 2% phosphorus and 5.8% potassium was also sprayed in two crates of the donor plants one week before taking the leaf cuttings. The concentration applied was 5ml per 1.5/litre of water. Two crates of donor plants were used per cultivar per treatment. Calcium nitrate and potassium nitrate (in the second season) was applied one week before the cuttings were taken.

Leaves were severed randomly and planted in decomposed bark. Eight replications of 30 cuttings each of distal and proximal sections of leaves were planted.

Variables that were examined are:

- a) number of bulblets that were produced per leaf cutting,
- b) mass of bulblets,
- c) average size of bulblets and survival percentage was examined in the second season. Survival percentage was calculated by counting the number of surviving cuttings divided by the number of cuttings planted multiplied by 100.

Bulblets were harvested and data collected was analyzed as in Chapter 4.

### 6.3 Results and discussion.

Both cultivars did not respond positively to supplementary fertilization of the donor plants in the survival of leaf cuttings and number of bulblets. Cultivar Ronina produced more bulblets than Robyn did and Robyn produced many bulblets and higher mass (Figure 6.2) than Ronina. This difference was however, not due to fertilization treatments but to cultivar differences. Distal leaf sections of cultivar Robyn had better survival ability than that of Ronina. The difference in cultivar performance was not statistically significant (Table 3 A i, appendix). As in Chapter 4, the proximal leaf sections performed better than their distal counterparts in all variables examined.

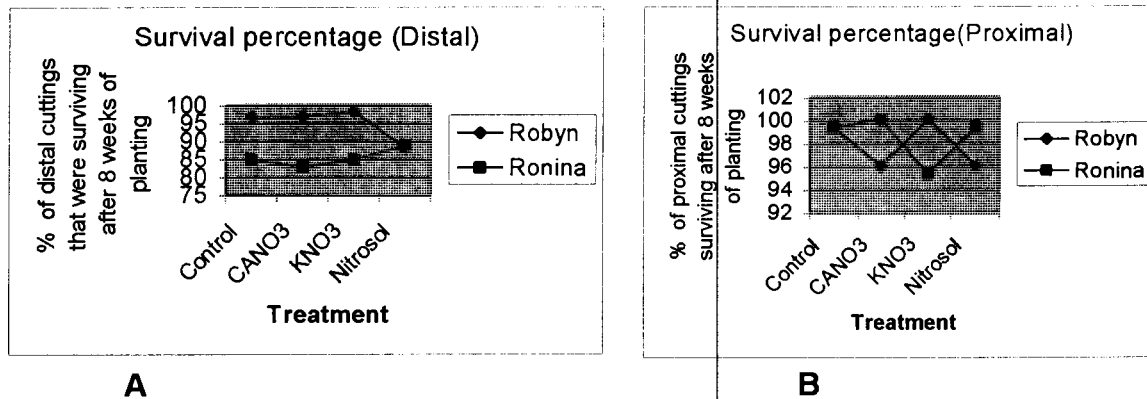


Figure 6.1: Effect of supplemented fertilization of mother bulbs (two weeks before taking cuttings) on the survival rate of leaf cuttings.

A: % survival of distal cuttings

B: % survival of proximal cuttings

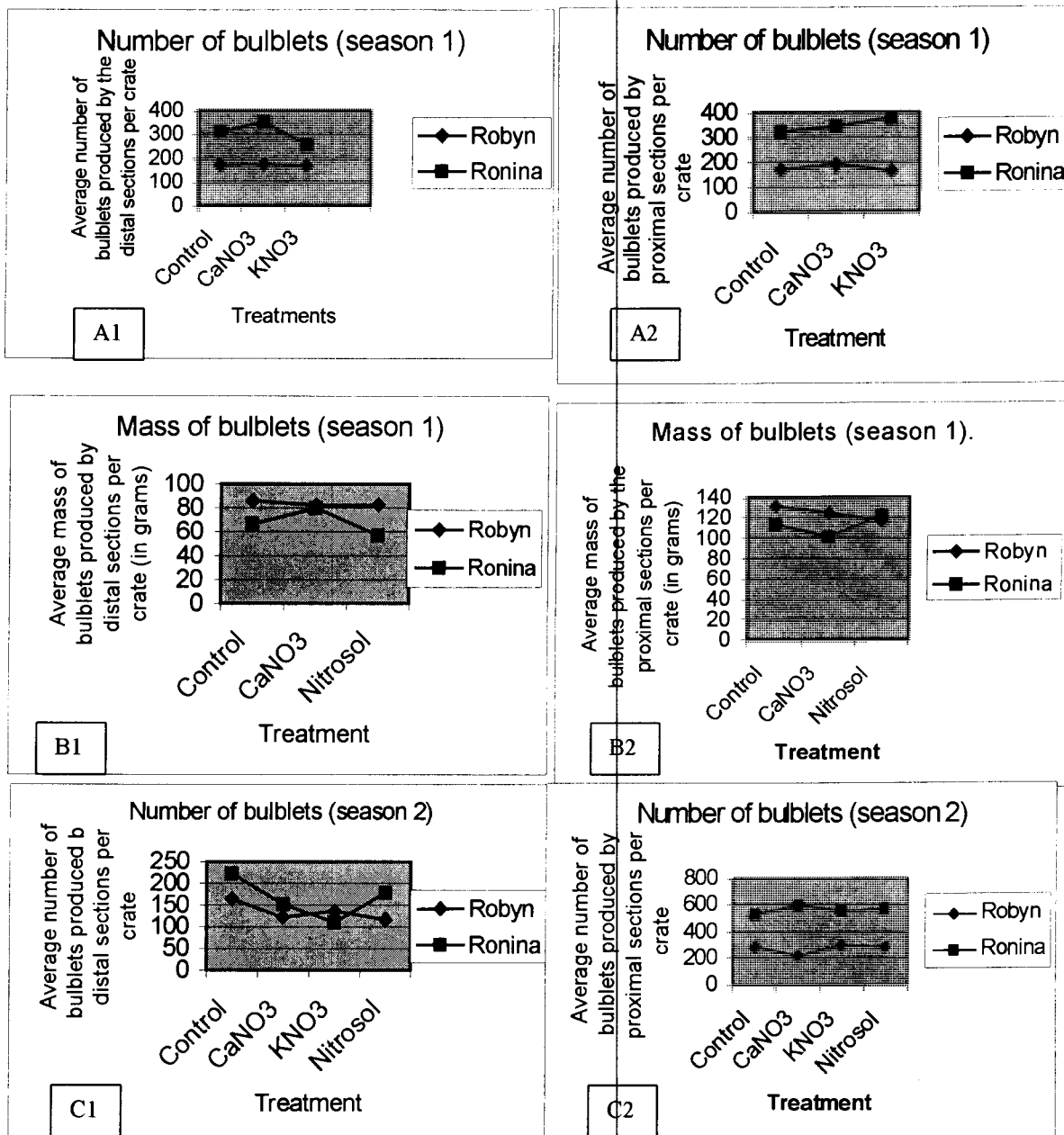


Figure 6.2: Effect of supplementary feeding of the mother plants on leaf cutting performance. A1, A2 and C1, C2 represent the number of bulbets produced in season 1 & 2 respectively, while B1 and B2 represent the mass of bulbets produced per crate in season 1.

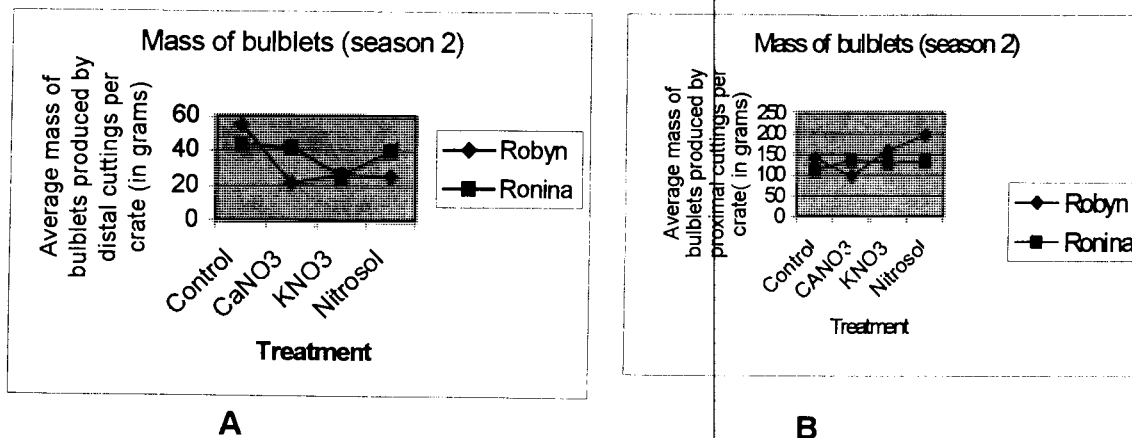


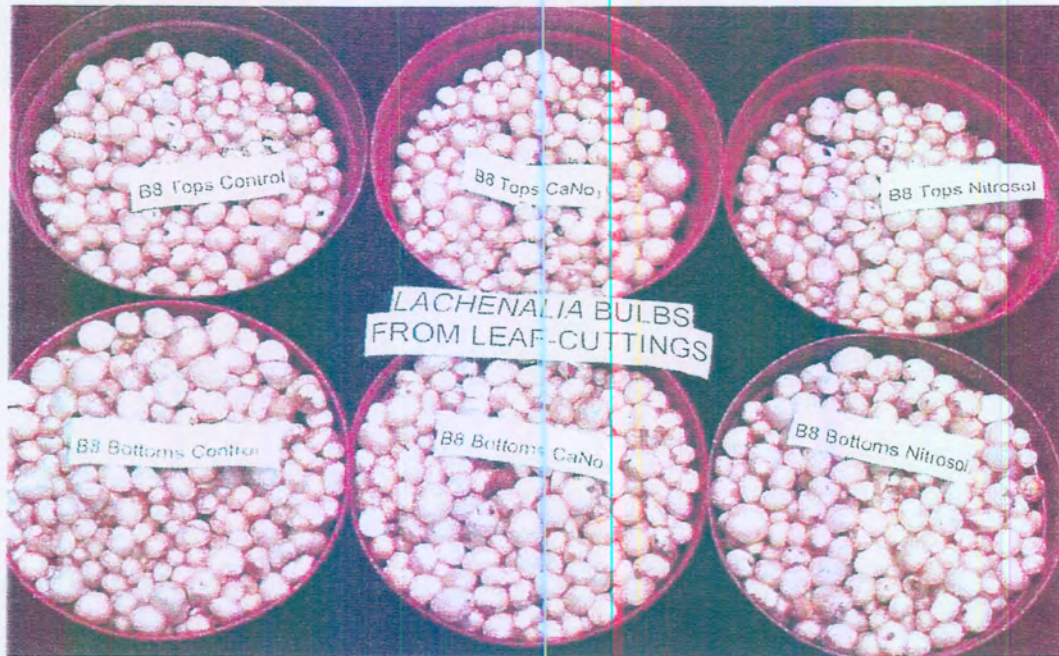
Figure 6.3: Effect of supplementary feeding of the mother plants on mass of the bulblets produced by leaf cuttings.

A: Average bulblet mass of distal cuttings

B: Average bulblet mass of proximal cuttings.

In the second season, the control gave the highest number of bulblets and mass in both cultivars (Figure 6.2 and 6.3). A slight increase was observed on the number of bulblets produced by proximal leaf sections in the first season (Figure 6.2), however the increase was not statistically significant.





A



B

**Figure 6.3:** *Lachenalia* bulblets produced by leaf cuttings after application of different nutrients. A: Robyn and B: Ronina (Season 1).

Data presented in this investigation suggest that there is no need for supplementary fertilizing the donor plants before taking leaf cuttings, as there was no significant difference in the percentage regeneration and number of bulblets. However, this might be due to the concentration used or the time of application and thus further research might be necessary. These results at this stage, however, support the findings of Duncan (1988) that *Lachenalia* species can be grown successfully without any supplementary fertilization of bulbs, though in this investigation, Osmocote and lime was added to sand. Looking at the current production of bulblets by leaf cuttings (Table 5.1, appendix) further research on time of application and concentration is however, not necessary.

There was no significant difference between cultivar Robyn and Ronina in the survival of leaf cuttings (Table 5.1) but Ronina produced significantly more bulblets per cutting than Robyn but they were significantly smaller (Table 5.1, appendix). However there was no significant difference in mass of the bulblets produced by these two cultivars (Table 5.1). There was a significant difference in the size of the bulblets produced by these two cultivars (Table 5.1, appendix). The proximal cuttings outperformed their distal counterparts in all four variables (regeneration, number, mass and size of bulblets) examined in this trial. There was no significant difference between the supplementary fertilization treatment in the regeneration and number of bulblets produced (Table 3 Ai and 3 Bi, appendix).

## 6.4 Conclusion.

Looking at the current leaf cutting production and the results of this investigation, there seems to be no advantage in supplementary fertilizing unless it is done at an earlier stage. The time of fertilizing and the concentration might have played a role in these results but further research seems to be necessary at this stage.

Considering the cost of  $\text{CaNO}_3$ ,  $\text{KNO}_3$  and Nitrosol, it is not recommend as it is not economically feasible to fertilize the donor plants before taking the leaf cuttings in the current situation.

## CHAPTER 7

# EFFECT OF DISINFECTANTS ON *LACHENALIA* LEAF-CUTTINGS PERFORMANCE.

### 7.1 Introduction.

It has been observed that cuttings that live longer tend to produce good quality bulblets (Chapter 4). In order to prevent loss of the leaf cuttings by rotting and other fungal attack, disinfectants were used in this investigation. The use of disinfectants on *Lachenalia* leaf-cuttings has not been tested. Preservatives enhance the uptake of water and prevent bacterial and fungi infection in cut flowers. When the best combination of preservatives or disinfectants are used in cut flowers, vase life can be increased two to three times (LARSON, 1992).

The objective of this chapter was to study the effect of disinfectants including some that are used in cut flowers on the performance of *Lachenalia* distal leaf cuttings. As observed in Chapter 4, proximal leaf sections performed better than distal sections in all variables examined, and for this reason only distal sections were used in this trial to test if their performance can be improved by applying disinfectants.

## 7.2 Materials and method.

The distal leaf cuttings of cultivar Robyn were used in this experiment. Mature bulbs were planted under a shade structure (55% irradiance) to obtain healthy leaves. Cuttings were planted under similar conditions as those of Chapter 4. Five treatments were applied, namely; control (water was applied), HTH (a granular dry chlorine containing calcium hypochlorite with 70% available chlorine applied at concentration of 5g/litre), Sporkil (a cut flower disinfectant and the concentration used was 1ml/litre) and Fongarid<sup>tm</sup> (a wettable powder systemic fungicide for the control of root and stem rot and damping off caused by *Pythium* and *Phytophthora* in citrus and ornamental nurseries). The dosage used was 5ml per crate and was applied as a drench after planting the cuttings in decomposed bark. Each treatment was replicated five times. Thirty (30) cuttings per replication were used in this experiment. The trial was repeated the following year.

Criteria that were evaluated were:

- i) Survival percentage (viability) of leaf-cuttings per crate after eight weeks of planting,
- ii) Quantity (number) of bulblets per crate, and
- iii) Mass of bulblets and size (second season) produced per crate.

Data was collected and analyzed as in chapter 4.

### 7.3 Results and discussion.

The control gave the highest number of survived leaf cuttings after eight weeks of planting and the highest number of bulblets in both two seasons (Figure 7.1 and 7.3). Sporkil™ seem to have a negative effect on leaf cuttings as it gave the lowest survival rate in both two seasons.

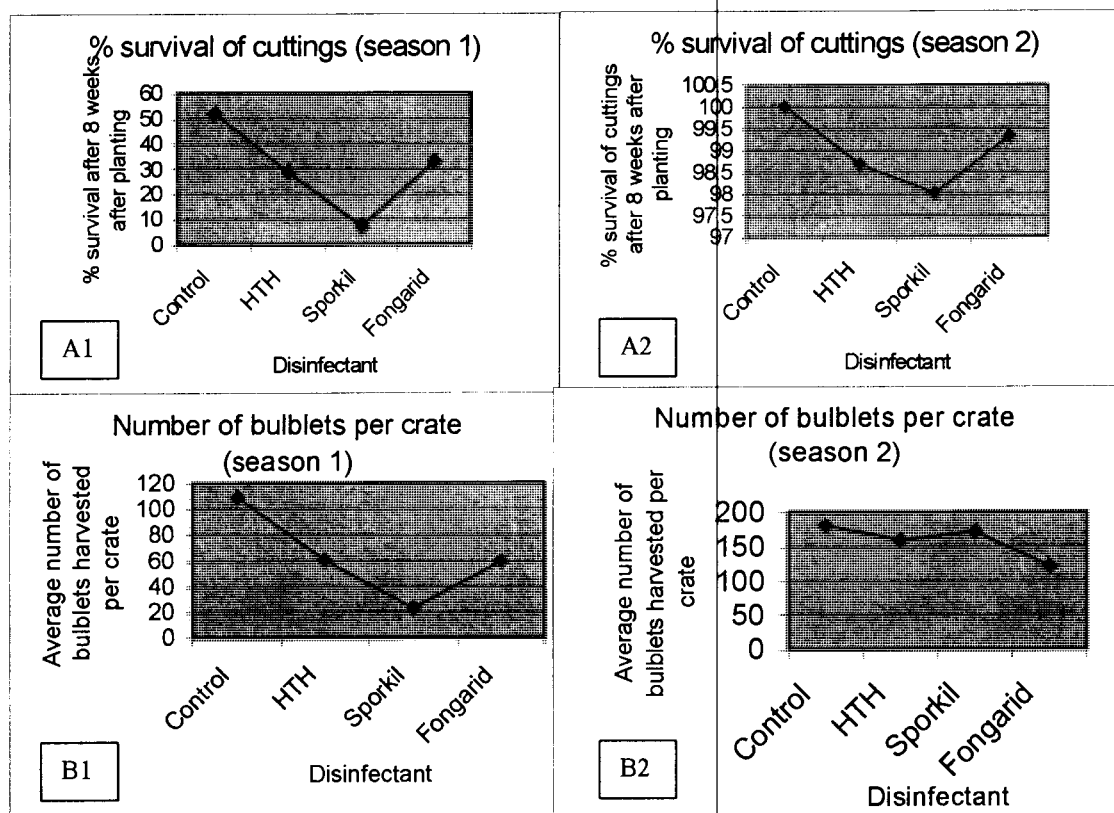


Figure 7.1: Effect of Disinfectants on *Lachenalia* leaf cuttings survival rate and number of bulblets produced (cultivar Robyn). A1 and A2: % survival rate of leaf cutting/crate in season 1 & 2 respectively. B1 and B2: number of bulblets harvested per crate in season 1 & 2 respectively.

The control gave a statistically significant higher (Figure 7.2) mass and number of bulblets in the first season as compared to other treatments (Figure 7.3). The results of this investigation also supports the findings of Chapter 4 that leaf cuttings taken earlier had higher survival ability than cuttings taken late in the season (Figure 7.1 A1 and A2).

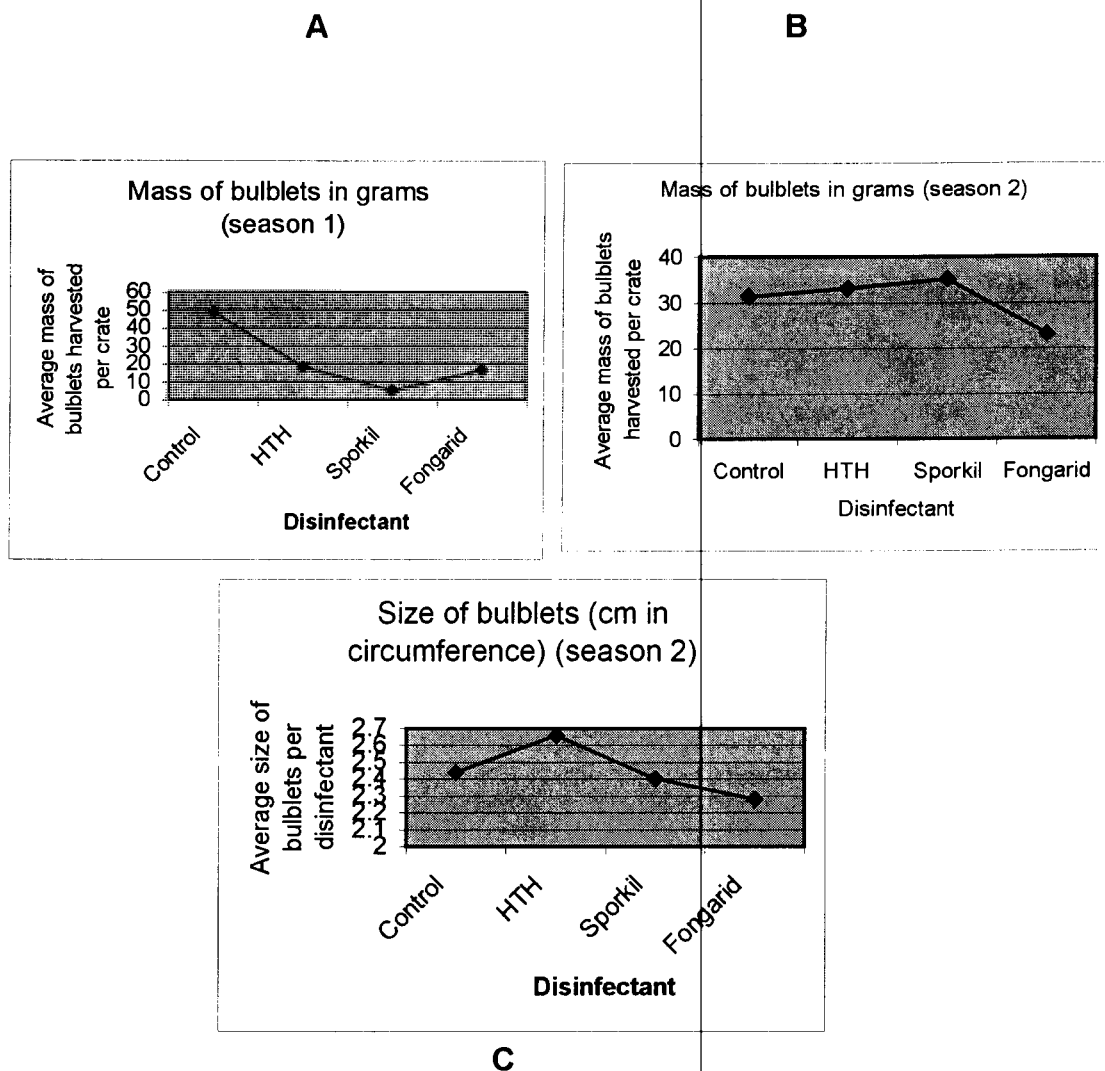


Figure 7.2: Effect of Disinfectants on the mass and size of bulblets produced by leaf cuttings. A & B: Average mass of bulblets per crate for season 1 & 2 respectively. C: average size of bulblets harvested from distal leaf cuttings.

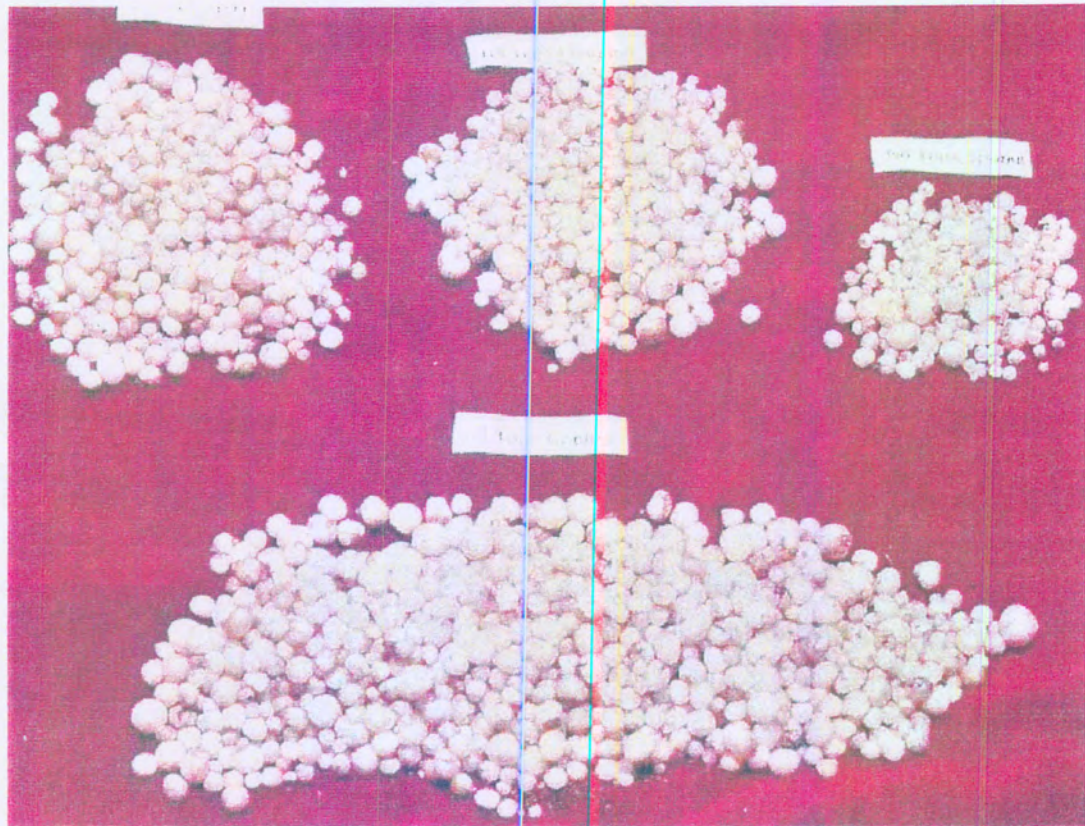


Figure 7.3: *Lachenalia* bulblets harvested from the distal leaf sections of cultivar Robyn treated with disinfectants as indicated in Figure.

Higher survival rate was observed in the second season (Figure 7.1), this was however not due to disinfectant treatment but do to the fact that leaf cuttings were taken earlier in the season due to early sprouting of the mother bulbs in the second season. The number of bulblets followed the trend of the survival ability of leaf cuttings in both seasons. This is of importance to the grower as high number of survived cuttings led to high number of bulblets produced in this investigation (Figure 7.1).



Though in the second season the control gave a lower mass, there was no significant difference compared with other treatments. HTH gave the biggest bulblets (Figure 7.2) compared to other treatments, however, the difference was not significant. There was a significant variation between disinfectant treatments in the survival rate (Table 2Ai), number of bulblets (Table 2Bi) and mass of bulblets (Table 2 Ci, appendix) in the first season.

The data found in this trial gave the impression that disinfectants do not have a beneficial effect on *Lachenalia* leaf cuttings. As leaf cuttings were taken and planted earlier in the second season, planting earlier had a positive effect on the performance of leaf cuttings as higher percentage of cuttings survived were obtained in this investigation and bigger bulblets are harvested.

#### **7.4 Conclusion.**

Data obtained in this investigation suggest that there is no beneficial effect to apply disinfectants on *Lachenalia* leaf-cuttings.

Considering costs of disinfectants, labour and time; one cannot recommend applying preservatives on *Lachenalia* leaf cuttings as the untreated cuttings outperformed those treated with disinfectants (Figure 7.3).

## CHAPTER 8

# STARCH DEPOSITION ON *LACHENALIA* LEAVES AND BULBS DURING THE GROWING SEASON.

### 8.1 Introduction.

Starch is a major carbohydrate in plants (Miller, 1992a). The starch concentration in geophytes is highly variable between species and tissues, and highly dependent on environmental conditions (Miller, 1992a). During early shoot growth when stored reserves are utilized, it is expected that the starch content of the storage organ will decrease, and subsequently increase after anthesis when filling is most rapid. Starch deposition in leaves and bulbs of *Lachenalia* has not yet been studied.

The objective of this chapter was to assess starch deposition in *Lachenalia* leaves and bulb during the growing season and to investigate any possible relationship between starch deposition and leaf cutting performance.

### 8.2 Materials and methods.

Leaf samples were taken in two physiological stages, namely: before flowering and at full flowering for the first year. For the second year, samples were taken in

all three physiological stages studied in Chapter 4. The cultivars used in this trial were 'Romelia', 'Robyn' and 'Rupert' in the first year where as the first two were used in the second year. Leaves were severed every hour from 08h00 to 20h00 in the first season while leaf sections were fixed hourly for 24 hours during the second season. The bulb was dissected during the day. The samples that were severed from the bulb were outer-scale, inner-scale (green leaf bases), basal plate, inflorescence stalk and the young of the inflorescence.

For Light microscopy, samples were fixed in gluteraldehyde (GA) plus buffer (0.15 MP). Samples were embedded in Glycol methacrylate II (GMA) and cut (2-5 $\mu$ ) and sections mounted on slides.

### Staining.

PERIODIC ACID-SCHIFF (PAS) STAIN (Feder and O'Brien, 1968).

Sections mounted on slides were fixed in aldehyde and rinsed with DNPH. Slides were placed in 1% periodic acid for five to ten minutes They were washed in running water for five to ten minutes, placed in Schiff's reagent for ten to thirty minutes, and rinsed in running water for five to ten minutes. Slides were dried on a hot plate before they were stained with toluidine blue, rinsed in running water, and were again put on a hot plate to dry out before observing them.

Transmission electron microscope (TEM).

For TEM observation, samples were fixed in GA, embedded in GMA, were put in an oven to polymerize. Sections were cut and mounted on grid, stained with Uraniel acetate and Pb citrate after which they were ready for TEM investigation.

### 8.3 Results and observations.

In the leaf-sections analyzed both with TEM and PAS staining in this investigation, starch was not observed (Figure 8.1 A). Starch was observed in all bulb sections: the outer-scale, inner-scale (green leaf bases), basal plate and developing inflorescence. More starch grains were observed in the sections from the developing inflorescence stalk (Figure 8.1 B). It is tempting to say there is little or no starch in *Lachenalia* leaves. The reason for the absence of starch in the leaves could be that starch formed in the leaves are immediately converted into sugars and translocated to the bulb scales for storage. These results are however contradicting the findings of Niederwieser (1990) that starch grains were clearly visible in leaf sections grown *in vitro*, but it must be emphasized that the *in vitro* leaf sections had no sink.

In the leaves of *Lilium longiflorum* very few starch grains were observed, and sucrose appears to be the main storage sugar (Miller and Langhans, 1989). No test for sucrose was however, done for *Lachenalia*. Observations on *Lilium*

*longiflorum* leaf sections stained with iodine, revealed heavy staining in guard cells with little staining leaf mesophyll cells (Miller, 1992a).

According to Chen (1966), leaves of *Narcissus* also contain very little starch; but heavy iodine staining was observed on *Narcissus* bulbs and in developing flowers.

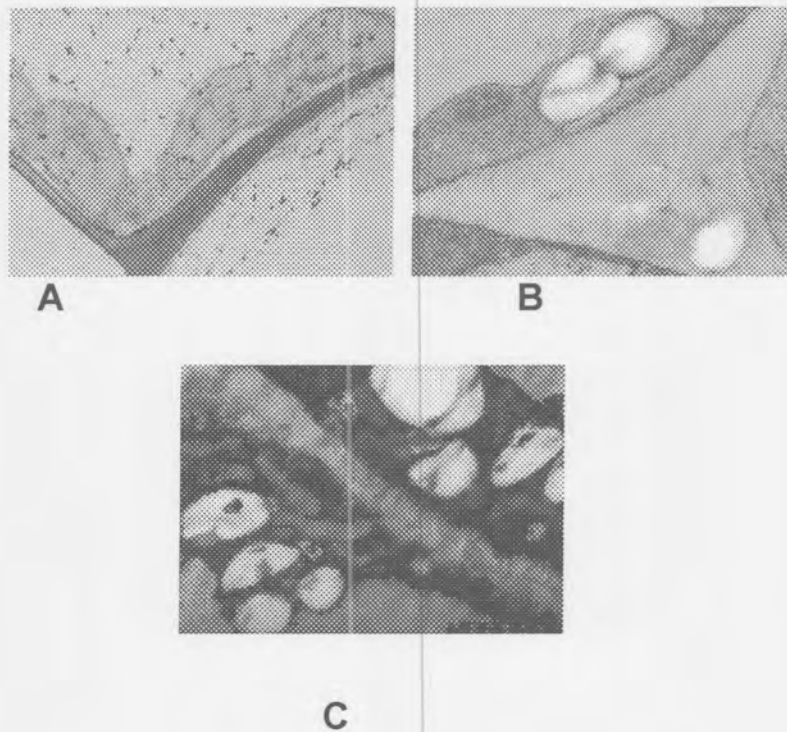


Figure 8.1: Ultramicrographs of bulb tissue showing:

- A: Chloroplasts in leaf sections with no starch of section fixed at 10:00am.
- B: Sparse starch grains occurring in a section of green leaf base.
- C: Starch grains in the section from the inflorescence stalk.

More starch grains were observed in developing flowers for *Lachenalia* in this investigation than in bulb scales. In *Narcissus tazetta*, starch was abundantly stored in all storage organs (bulb parts) although the level was relatively uniform in the green leaf bases under high light intensity (Chen, 1969). Similar results were obtained for *Lachenalia* bulb scale sections in the sense that they also contained starch grains.

The latter author found that starch also occurred in both the bundle sheaths throughout the whole leaf blade and in guard cells. According to Chen (1969), the presence of starch cannot be detected by ordinary Sach's test, but the blade sections must be stained directly with iodine solution. This method should be tried for *Lachenalia* in future research.

In *Narcissus* sucrose was continuously translocated from the leaf blade and scape into their bases where it was stored (Chen, 1969). The latter author again reported that sucrose decreased in the blade and the green part of the scale in the dark, but increased as light was increased. At the time of the above ground senescence, sugar disappeared almost completely (Chen, 1969).

Chen (1969) concluded that sucrose is the first formed sugar of photosynthesis in *Narcissus* leaf and the free hexose sugar present in cells does not participate in sucrose or starch synthesis.

#### 8.4 Conclusion.

More starch is concentrated in the bulb in *Lachenalia* as all bulb parts examined in this investigation had starch. Starch was not observed in leaf sections examined with both TEM and under light microscope after PAS staining. Young developing inflorescence parts inside the bulbs have the most starch (Figure 8.1) as compared to other bulb parts.

Leaves of both *Lilium longiflorum* (Miller and Langhans, 1989) and *Narcissus* (Chen, 1966) have very little starch although heavy iodine staining for starch was observed in developing flowers. The same trend might prevail for *Lachenalia*. According to the observations done by cited authors on other members of the bulbous Hyacinthaceae, there seems to be a great deal of variability regarding storage products in the green leaves and bulbs. A proper review and additional research is necessary.

## CHAPTER 9

### GENERAL DISCUSSION AND RECOMMENDATIONS.

Optimization of bulblet production by leaf cuttings of *Lachenalia* can be achieved if factors that affect the performance of leaf cuttings are observed and addressed and those that were thought to be important were studied in this investigation. It was observed in this study that if bigger and more bulblets need to be produced, leaf sections should be cut from the base of leaves when the inflorescence is visible between the leaf sheaths.

Whole leaf sections can be used if bigger bulblets need to be harvested but if many bulblets of medium size (2-3cm in circumference) need to be produced, leaf cuttings can be severed and divided into the distal and proximal cuttings at the stage when the inflorescence is visible between leaf sheaths. In this investigation the growing medium that gave the best results was a commercial decomposed pine bark.

The results of this investigation showed that proximal sections performed better than their distal counterparts in all variables (survival ability, number, size and mass of bulblets) evaluated but the difference became more evident as leaves of the donor plant matured. It is therefore not recommended to take distal cuttings when the donor plants are in full flowering. It is apparent that the most important aspect is the age of the leaf tissue that is actually determining the performance



and the physiological stage of the inflorescence is merely an indicator for leaf tissue maturity. At the time when the inflorescence is visible between the leaf sheath, the intercallary meristem at the junction of the leaf base and lamina is still active, and therefore contains immature, totipotent tissues.

Fertilizing the donor plants before taking the leaf cuttings and applying disinfectants had no beneficial effect on bulblet formation on *Lachenalia* leaf cuttings in this study. Considering the present production of bulblets by leaf cuttings and the costs of disinfectants and fertilizers, it cannot be recommended to apply disinfectants when planting the distal cuttings and to fertilize the donor plants before taking leaf cuttings.

The most important aspect of propagating *Lachenalia* by leaf cuttings is the age of the plant at which leaf cuttings are taken. When senescence time arrived, cuttings died down at the same time no matter which physiological stage they were severed from the donor plants. Leaf cuttings should be taken earlier in the growing season to give them enough time to support the bulblets.

Bulblets on *Lachenalia* leaf cuttings are of exogenous origin and developed as a result of the division of epidermal cells. Roots on leaf cuttings originated endogenously from the parenchyma cell associated with the vascular tissues while roots on bulblets develop from the base of the meristematic mass of cells which form the bulblets, and are therefore attached to the bulblets.

## CHAPTER 10

### IMPLICATION ON SMALL SCALE FARMERS

Propagating *Lachenalia* by leaf cuttings is an easy way of propagation. Small-scale farmers can also afford to do it, as there is no expensive chemicals, equipments and/or infrastructure needed like in tissue culture propagation. Leaf cuttings are planted under a shade structure, even where the temperature is not controlled and still yield very well.

This method of propagation also provide job opportunities for people as it is labor intensive. A RDP project for growing *Lachenalia* was established in Nieuwoudtville (situated on the edge of Namaqualand and Bokkeveld region in the Northern Cape Province). This crop was selected because the species grow naturally in the wild in this region.

*Lachenalia* is endemic and developed in South Africa and thus propagation and growing bulbs is still done only in South Africa. These gives people the opportunity to grow *Lachenalia*, but only those granted licenses. The ARC-Roodeplaat had already released quite a number of cultivars of which growers can choose from. It should be noted that some cultivars are easier (e.g. Romelia) to propagate than others (like Rupert) as the results of this investigation had shown. Farmers need to know when to take leaf cutting and which medium to use for leaf cuttings.

At the present moment, the demand for *Lachenalia* bulbs is estimated at 20 million bulbs per annum in Europe only and this might give the opportunity to small-scale farmers to grow it. It should be understood that the grower has to know various aspects on its production and the results of this investigation are of importance in that regard.

The only problem for now is that the market has not yet been developed in South Africa. Assessment on the market had been done and the results were that the market is small in South Africa as compared to Europe and America (Niederwieser *et al.*, 1997).

Most small-scale farmers grow vegetables in South Africa and the market for vegetables is higher than that of pot plants like *Lachenalia*. The demand for flowers was found to be high on specific dates like Valentine day, Mothers day and during Easter.

**APPENDIX**

SAMMARY OF ANALYSIS OF VARIANCE. (For abbreviations refer to page iv.)

**Table 1A** Effect of physiological stage on the survival of leaf cuttings.

Source	DF	SMS	MS	FV	PR>F
Error	72	1340.80	18.62		
T	89	52595.66			
CV	2	776.82	388.41	20.86	0.0001**
S	2	970.82	485.41	26.07	0.0001**
P	1	46648.90	46648.90	2505.01	0.0001**
CV*S	4	1213.11	303.27	16.29	0.0001**
CV*P	2	594.06	297.03	15.95	0.0001**
S*P	2	88.47	44.23	2.38	0.0001**
CV*S*P	4	962.67	240.67	12.92	0.0001**
R-Square		CV			
0.97		12.57			

**Table 1B** Effect of physiological stage on the number of bulblets produced by cuttings.

Source	DF	SMS	MS	FV	PR>F
Error	72	59294.00	8323.53		
Total	89	4303625.79			
CV	2	1305523.62	65761.81	78.42	0.0001**
S	2	164385.48	82192.74	9.87	0.0001**
P	1	1636741.88	1636741.88	196.64	0.0001**
CV*S	4	21094.78	5273.69	0.63	0.6402 NS
CV*P	2	480004.28	240002.14	28.83	0.0001**
S*P	2	79361.35	39680.68	4.77	0.0114*
CV*S*P	4	17220.38	4305.09	.52	0.7233 NS
R-square		CV			
0.86		46.92			

**Table 1C** Effect of physiological stage on mass of bulblets harvested from cuttings.

Source	DF	SMS	MS	FV	PR>F
Error	72	38902.64	540.31		
Total	89	315179.86			
CV	2	1449.97	724.99	1.34	0.2678NS
S	2	71258.00	35629.00	65.94	0.0001**
P	1	132354.28	132354.28	244.95	0.0001**
CV*S	4	9996.40	2499.09	4.63	0.0001**
CV*P	2	105.67	52.84	0.10	0.9070 NS
S*P	2	49206.76	24603.38	45.54	0.0001**
CV*S*P	4	11906.12	2976.53	5.51	0.0001**
	R-square	CV			
	0.88	50.17			

**Table 1D** Effect of physiological stage on size of bulblets harvested from cuttings.

Source	DF	SMS	MS	FV	PR>F
Error	72	13.33	0.19		
Total	89	95.24			
CV	2	6.60	3.30	17.82	0.0001**
S	2	32.06	16.03	86.59	0.0001**
P	1	18.758	18.75	101.26	0.0001**
CV*S	4	16.07	4.01	21.70	0.0001**
CV*P	2	6.93	3.46	18.73	0.0001**
S*P	2	0.56	0.28	1.52	0.2260NS
CV*S*P	4	0.91	0.23	1.24	0.3037NS
	R-square	CV			
	0.86	21.43			

**Table 2A (i)** Effect of disinfectants on the survival of leaf cuttings (Season 1).

Source	DF	SMS	MS	FV	PR>F
Error	36	342.80	9.52		
Total	39	975.90			
D	3	633.10	211.03	22.16	0.0001**
R-square		CV			
0.65		41.42			

**Table 2A (ii)** Effect of disinfectants on the survival of leaf cuttings (season 2)

Source	DF	SMS	MS	FV	PR>F
Error	16	9.20	0.58		
Total	19	10.20			
Model	3	1.00	0.33	0.58	0.6368 NS
R-square		CV			
0.10		0.76			

**Table 2B (i)** Effect of disinfectants on the number of bulblets harvested from cuttings (Season 1)

Source	DF	SMS	MS	FV	PR>F
Error	36	13639.50	378.88		
Total	39	51026.78			
Treat	3	37387.28	12462.43	32.89	0.0001**
R-square		CV			
0.73		31.23			

**Table 2B (ii)** Effect of disinfectants on the number of bulblets harvested from cuttings (Season 2)

Source	DF	SMS	MS	FV	PR>F
Error	16	24695.20	1543.45		
Total	19	34756.55			
D	3	10061.35	3353.78	2.17	0.1311 NS
	R-square	CV			
	0.29	25.08			

**Table 2C (i)** Effect of disinfectants on mass of the bulblets harvested from leaf cuttings (Season 1)

Source	DF	SMS	MS	FV	PR>F
Error	36	3328.72	92.47		
Total	39	13433.38			
D	3	10104.66	3368.22	36.43	0.0001**
	R-square	CV			
	0.75	44.15			

**Table 2C (ii)** Effect of disinfectants on the mass of the bulblets harvested from cuttings (Season 2)

Source	DF	SMS	MS	FV	PR>F
Error	16	1120.22	70.01		
Total	19	1543.91			
D	3	423.69	141.23	2.02	0.1521NS
	R-square	CV			
	0.27	27.26			

**Table 2D (i)** Effect of disinfectants on the size of bulblets harvested from cuttings (Season 1)

Source	DF	SMS	MS	FV	PR>F
Error	36	1.53	0.04		
Total	39	15.14			
D	3	13.60	4.54	106.44	0.0001**
	R-square	CV			
	0.90	12.51			

**Table 2D (ii)** Effect of disinfectants on the size of bulblets harvested from cuttings (Season 2)

Source	DF	SMS	MS	FV	PR>F
Error	16	0.83	0.05		
Total	19	1.20			
D	3	0.38	0.13	2.42	0.1040NS
	R-square	CV			
	0.31	9.33			

**Table 3A (i)** Effect of fertilization of mother bulbs on the survival of cuttings (Season 2)

Source	DF	SMS	MS	FV	PR>F
Error	64	1092.00	17.06		
Total	79	1345.95			
CV	1	36.45	36.45	2.14	0.1487NS
P	1	120.05	120.05	7.04	0.0101*
F	3	4.05	1.35	0.08	0.9711NS
CV*P	1	48.05	48.05	2.82	0.0982NS
CV*F	3	29.25	9.75	0.57	0.6359NS
P*F	3	2.25	0.75	0.04	0.9876NS
CV*P*F	3	13.85	4.62	0.27	0.8464NS
	R-square	C.V.			
	0.19	14.63			



**Table 3B (i)** Effect of fertilization of the mother bulbs on the number of bulblets harvested from cuttings (Season 1)

Source	DF	SMS	MS	FV	PR>F
Error	84	249263.25	2967.42		
Total	95	902705.83			
CV	1	576290.04	576290.04	194.21	0.0001**
F	2	10233.33	5116.67	1.72	0.1846NS
P	1	13207.04	13207.04	4.45	0.0379*
CV*F	2	1914.08	957.04	0.32	0.7252NS
CV*P	1	8362.67	8362.67	2.82	0.0969NS
P*F	2	16691.08	8345.54	2.81	0.0657NS
CV*P*F	2	26744.33	13372.17	4.51	0.0138*
R-square		C.V.			
0.72		22.07			

**Table 3B (ii)** Effect of fertilization of the mother bulbs on the number bulblets harvested from cuttings (Season 2).

Source	DF	SMS	MS	FV	PR>F
Error	64	182842.80	2856.92		
Total	79	2451738.00			
CV	1	515526.05	515526.05	180.45	0.0001**
P	1	1348362.45	1348362.45	471.96	0.0001**
F	3	102107.50	3402.50	1.19	0.3203 NS
CV*P	1	335923.20	335923.20	117.58	0.0001**
CV*F	3	20567.65	6855.68	2.40	0.0760NS
P*F	3	21194.85	7064.95	2.47	0.0696NS
CV*P*F	3	17113.50	5704.50	2.00	0.1233NS
R-square		C.V.			
0.95		19.12			

**Table 3C (i)** Effect of fertilization of mother bulbs on the mass of the bulblets harvested from cuttings (Season 1)

Source	DF	SMS	MS	FV	PR>F
Error	84	27038.56	321.89		
Total	95	78898.31			
CV	1	4689.73	4689.73	14.57	0.0003**
F	2	420.13	210.06	0.65	0.5233NS
P	1	42238.62	42238.62	131.22	0.0001**
CV*F	2	307.47	153.74	0.48	0.6219NS
CV*P	1	78.88	78.88	0.25	0.6219NS
P*F	2	1412.73	706.37	2.19	0.0180*
CV*P*F	2	2712.20	1356.10	4.21	0.0180*
R-square		C.V.			
0.66		18.59			

**Table 3C (ii)** Effect of fertilization of mother bulbs on the mass of bulblets harvested from cuttings (Season 2).

Source	DF	SMS	MS	FV	PR>F
Error	64	28895.44	451.49		
Total	79	268810.72			
CV	1	1401.05	1401.05	3.10	0.0829NS
P	1	203018.30	203018.30	442.66	0.0001**
F	3	5840.50	1946.83	4.31	0.0078*
CV*P	1	3962.25	962.25	8.78	0.0043*
CV*F	3	9217.50	3072.50	6.81	0.0005**
P*F	3	10490.96	3498.32	7.75	0.0002**
CV*P*F	3	5980.72	1993.57	4.42	0.0069*
R-square		C.V.			
0.89		25.11			

**Table 3D (i)** Effect of fertilization of the mother bulbs on the size of bulblets harvested from cuttings (Season 2).

Source	DF	SMS	MS	FV	PR>F
Error	64	4.56	0.07		
Total	79	49.99			
CV	1	21.22	21.22	298.06	0.0001**
F	1	9.80	9.80	137.66	0.0001**
P	3	2.82	0.94	13.19	0.0001**
CV*F	1	7.32	7.32	102.83	0.0001**
CV*P	3	3.06	1.02	14.36	0.0001**
P*F	3	0.86	0.29	4.01	0.0112*
CV*P*F	3	0.37	0.12	1.71	0.1735NS
R-square	0.90	C.V.	9.35		

**Table 4A (i)** Effect of growing medium on the survival of cuttings (Season 2).

Source	DF	SMS	MS	FV	PR>F
Error	16	163.60	10.23		
Total	19	588.55			
M	3	424.95	141.65	1385	0.0001**
R-square	0.72	C.V.	12.71		

**Table 4B (i)** Effect of growth medium on the number of bulblets harvested from cuttings (Season 2)

Source	DF	SMS	MS	FV	PR>F
Error	16	21859.20	1366.20		
Total	19	59991.20			
M	3	38132.00			0.0009**
R-square	0.64	C.V.	29.52		

**Table 4C (i)** Effect of growing medium on the mass of bulblets harvested from cuttings (Season 2).

Source	DF	SMS	MS	FV	PR>F
Error	16	3506.19	219.14		
Total	19	14872.15			
M	3	11365.97	3788.66	17.29	0.0001**
	R-square	C.V.			
	0.76	28.88			

**Table 4D (i)** Effect of growing medium on the size of bulblets harvested from cuttings (Season 2).

Source	DF	SMS	MS	FV	PR>F
Error	16	3.22	0.20		
Total	19	5.86			
M	3	2.64	0.88	4.37	0.0199*
	R-square	C.V.			
	0.45	13.91			

**Table 5.1:** Effect of cultivar and position on survival, number, mass and size of bulblets harvested from cuttings. Means within a column followed by the same letter are not significantly different ( $P=0.05$ ) according to Duncan's multiple range test.

Cultivar	Survival %	Average number of bulblets/cutting	Average mass of bulblets	Average size of bulblets
Robyn	96.33 a	6.89 a	12.89 a	3.37 a
Ronina	91.83 a	13.06 b	6.16 a	2.34 b
<b>Position</b>				
Distal	90.00 a	5.54 a	6.18 a	2.50 a
Proximal	98.17 b	13.91 b	9.70 b	3.20 b

## REFERENCES.

- BRYAN, J.E., 1989. *Bulbs*. Vol. 1, A-H. Timber Press, Portland, Oregon.
- BROWSE, P.M., 1979. *Plant Propagation*. The Royal Horticultural Society's Encyclopedia of Practical Gardening. Seeds, Roots, Bulbs and Corms, Layering, Cuttings, Budding and Grafting. Mitchell Beazley publishers limited, London.
- BULLETIN No. 197., 1967. HMSO 12s 6d net: *Flowers from bulbs and Corms*. Ministry of Agriculture, Fisheries and Food, London.
- CHEN, S., 1966. Localization of starch at the base of *Narcissus* leaves. *Annals of Botany*. **30**, 721-725.
- CHEN, S., 1969. Carbohydrate metabolism in the *Narcissus* leaf. *Journal of Experimental Botany*. **20**, 302-316.
- COOK, H., 1931. Propagation of *Lachenalia* by leaf-cuttings. *Journal of the Botanical Society of South Africa*. **12**, 12.
- CROSBY, T., 1986. The genus *Lachenalia*. *The Plantsman* **8**:129.

- DE HERTOIGH, A. & LE NARD, M., 1993. The physiology of flower bulbs. Elsevier Science Publishers. Amsterdam, London.
- DOERFLINGER, F., 1973. The Bulb book. The Pitman Press, Bath for David & Charles: Newton Abbot. London.
- DUNCAN, G., 1988. The *Lachenalia* handbook. Annals of Kirstenbosch, National Botanical Gardens, South Africa. Ed, J.N. Eloff.
- FAHN, A., 1982. Plant anatomy. 3<sup>rd</sup> edition. Pergamon Press, Pergamon.
- FEDER, N. & O'BRIEN, T.P., 1968. Plant microtechnique: Some principles and new methods. Biological laboratories, *American Journal of Botany*. **55**, 123-142.
- FOX, D., 1985. Growing Lilies. Croom Helm Ltd., Australia, Sydney.
- HANCKE, F.L. & LIEBENBERG, H., 1990. B-Chromosome in some *Lachenalia* species and hybrids. *South African Journal of Botany*. **56**, 659-664.
- HARTMANN, H.T. & KESTER, D.E., 1959. Plant Propagation. Principles and Practices. PRENTICE-HALL, Inc. New Jersey.

HARTMANN, H.T., KOFRANEK, A.M., RUBATZKY, V.E. & FLOCKER, W.J.,  
1988. Plant Science. Growth, development and utilization of cultivated  
plants, 2<sup>nd</sup> edition, Prentice Hall, New Jersey.

HARTMANN, H.T., KESTER, D.E. & DAVIES, F.T. Jr., 1990. Plant  
propagation. Principles and practices, 5<sup>th</sup> edition, Prentice-Hall, New  
Jersey.

HARTMANN, H.T., KESTER, D.E., DAVIES, F.T. Jr. & GENEVE, R.L., 1997.  
Plant propagation. Principles and practices, 6<sup>th</sup> edition. Prentice Hall,  
New Jersey.

HUSSEY, G., 1976. Plantlet regeneration from callus and parental tissue in  
*Ornithogalum thyrsoides*. *Journal of experimental Botany*. **27**, 375-  
382.

HUSSEY, G. & FALAVIGNA, A., 1980. Origin and production of *in vitro*  
adventitious shoots in the onion, *Allium cepa* L. *Journal of*  
*Experimental Botany*. **31**, 1675-1685.

KLESSER, P.J. & NEL, D.D., 1976. Virus diseases and tissue culture of some  
African bulbs. *Acta Horticulturae*. **59**, 71-76.

LARSON, R.A., 1992. Introduction to floriculture. 2<sup>nd</sup> edition. Academic Press, Inc. San Diego, California.

MACDONALD, B., 1972. Practical woody plants propagation for nursery growers. Vol. 1. Timber press, Portland, Oregon.

MAHLSTEDE, J.P. & HABER, E.S., 1957. Plant propagation. John Wiley & Sons, Inc. New York.

MILLER, W.B., 1992a. A Review of Carbohydrate Metabolism in Geophytes. *Acta Horticulturae*. **325**, 339-346.

MILLER, W.B., 1992b. Easter and hybrid Lily production. Principles and practice. Growers handbook series. Volume **5**. 12-14.

MILLER, W.B. & LANGHANS, R.W., 1989. Carbohydrate changes in Easter Lilies during growth in normal and reduced irradiance environments. *Journal of American Society of Horticultural Science*. **114**, 310-315.

MORI, G., HIRAI, H. & IMANISHI, H., 1997. Vegetative propagation of *Nerine* bulbs by crosscutting. *Acta Horticulturae*. **430**, 377-381.



- NAYLOR, E., 1940. Propagation of Hyacinthus by leaf cuttings. *Bulletin of the Torrey*. **67**, 602-606.
- NIEDERWIESER, J.G. & VCELAR, B.M., 1990. Regeneration of *Lachenalia* Species from leaf explants. *HortScience*. **25**, 684-687.
- NIEDERWIESER, J.G., 1990. Adventitious bud formation on *Lachenalia* Jacq. Leaf tissue *in vitro*. Ph.D. Thesis, University of Natal, Pietermaritzburg.
- NIEDERWIESER, J.G., MARASAS, C.N., ANANDAJAYASEKERAM, P., COETZEE, M., MARTELLA, D. & PIETERSE, B.J. 1997. Socio-economic impact of the *Lachenalia* research program. SACCAR, Gaborone, Botswana.
- PERRIGNON, R.J., 1992. Bulblet production *in vivo* from leaves of *Lachenalia*. *Acta Horticulturae*. **325**, 341-346.
- REES, A.R., 1972. The growth of bulbs. Applied aspects of the physiology of ornamental bulbous crop plant. Glasshouse Crops Research Institute. Littlehampton, Sussex, England. Academic Press, London.
- ROCKWELL, F.F. & GRAYSON, E.C., 1953. The complete book of bulbs. Garden city, American garden guild and Doubleday.

- RIX, E.M., 1983. Growing bulbs. Timber Press, Portland, Oregon.
- SANDER-ZIV, D, COHEN, A, KOCHIBA, I.M. EFRON, H., AMIT, D. & FINKELSTEIN, S., 1997. Improving *Hippeastrum* propagation and bulbil yield by cutting and incubation techniques. *Acta Horticulturae*. **430**, 355-360.
- SUH, J.K., ROH, M.S. & LEE, J.S., 1997. Leaf cutting propagation, growth and flowering of *Lachenalia*. *Acta Horticulturae*. **430**, 369-376.
- VAN LEEUWEN, P.J. & VAN DER WEIJDEN, J.A., 1997. Propagation of specialty bulbs by chipping. *Acta Horticulturae*. **430**, 351-353.
- VAN DER LINDE, P.C.G., 1992. Tissue culture of flower-bulb crops: Theory and Practice. *Acta Horticulturae*. **430**, 419-427.
- WITHAM FOGG, H.G.W., 1974. The complete handbook of bulbs. Ward Lock Limited, London.
- WALKER, R., 1940. Regeneration in the scale leaf of *Lilium candidum* and *L. longiflorum*. *American Journal of Botany*. **27**, 114-117.