EFFECTS OF GIBBERELLIC ACID AND BENZYLADENINE ON DORMANCY RELEASE, GROWTH AND MULTIPLICATION OF ORIENTAL LILY BULBS (*Lilium* spp)

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DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not been submitted wholly or in part for award of a degree in any university

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DEDICATION

To my parents Edwin and Beatrice Situma and siblings Sam, Stella, Nafula, Nekesa and for their encouragement, love and devotion.

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ABSTRACT

One of the major challenges facing lily cut flower production and marketing is high cost of production of the lily bulbs which undergo a dormancy period in their growth cycle as do other bulbous plants and for the growth to continue this dormancy has to be released. To mitigate this problem commercial growers apply artificial dormancy breaking using low temperatures (4-5° C) for 6-8 weeks. Low temperature treatments / cold storage of bulbs is expensive hence low cost dormancy breaking alternatives have to be sought. Studies done earlier have shown that Plant growth regulators can be used to break dormancy. Two trials were conducted at James Finlay Flower Company (Lemotit farm, Londiani), to evaluate the effects of gibberellic acid (GA_3) and benzyl adenine (BA) as alternative treatments for breaking dormancy in Oriental lily bulbs. The first trial was conducted from September 2009 to January 2010 while the second trial was conducted from January 2010 to April 2010. The factors were BA and GA_3 each at (0, 25, 50, 100, and 150) mg/l. Lily bulbs were soaked in different solution concentrations of BA and GA_3 at (0, 25, 50, 100, and 150)mg/l , singly and in combinations for 24 hours before planting. These treatments were compared with the conventional chilling treatment. Experiments were laid out in an unbalanced factorial design with three replications of 10 bulbs each and repeated in two trials. All data were subjected to analysis of variance (ANOVA) using the SAS statistical package. Significant means were separated using the Duncan's Multiples Range Test (DMRT). In both trials 50mg/l of BA and GA_3 singly significantly promoted higher sprout emergence, reduced the number of days to opening of the first leaf whorl, long stem lengths, long rachis, and reduced the number of days to flowering. BA at 100mg/l significantly accelerated the number of days to 50% sprout emergence, and promoted high numbers of leaves, florets, and bulblets. Significant combined effects of BA 100mg/l and GA_3 50mg/l were observed on the number of days to 50% sprout emergence, days to first leaf whorl opening, leaf count, long stems, increase in bulb weight, earlier flowering and highest number of bulblets. Combinations of the highest levels of the PGRs had adverse effects on all growth parameters resulting in dwarfed plants with poor quality flowers. BA and GA_3 both at 50mg/l singly and BA 100 mg/l plus GA_3 50 mg/l in combination proved to be the best treatments to replace chilling treatment for the growth and flowering of Oriental lily bulbs . It was concluded that low concentrations of BA and GA_3 can be used as alternative treatments to chilling for breaking dormancy in Oriental lily bulbs and furthermore leads to high yields and low cost of production hence economically viable for small scale farmers.

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LIST OF ABBREVIATIONS AND ACRONYMS

ABA	Abscisic acid
BA	Benzyl adenine
DMRT	Duncan's Multiple Range Test
GA_3	Gibberellic acid
HCDA	Horticultural Crops Development Authority
ILO	International Labour Organisation
mg/l	Milligrams per litre
SAS	Statistical Analysis Software
UNDP	United Nations Development Programme
PGRS	Plant growth regulators
WTO	World Trade Organisation
EU	European Union

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The horticulture sub-sector of agriculture which comprises of fruits, vegetables and flowers, is the fastest growing sub-sector of the Kenyan economy. It has grown extensively to become a major employer and foreign exchange earner; second only to remittances from the Kenyan Diaspora in 2008. Regardless of the difficult and challenging economic environment both at home and at the global level, the Kenya floriculture industry still experienced significant growth - from 91,000 tonnes in 2007 to 93,000 tonnes in 2008, fetching approximately Kshs 40 billion in foreign currencies.

The cut flower sector contributes more than 50% of the horticultural produce exported. Statistics indicate that in 2008 the export volume of floriculture was 3.31M metric tonnes, earning approximately 46.91 billion shillings (HCDA statistics report 2008). The floriculture industry according to 2004 estimates had approximately 5000 producers ranging from small scale to large scale operators. About fifty medium to large scale farms produce 75% of the total exports. The sizes of farms range from 20-100 hectares. About 10-15% of exports are produced by several small-to medium scale farms of 4-10 hectares. The remaining 5-13% of exports is produced by 3-4000 small holder farmers on less than 1.6 hectares, but mostly on a 0.41 hectares or less of land (Fintrac, 2005; Kolavalli, 2004). The floriculture industry employs between 50,000- 60,000 people directly, and 500,000 people indirectly. The sub-sector plays an important role in alleviation of poverty by providing increased income to the smallholder farmers who constitute about 75% of Kenya's farming population.

A flower is a plant considered with reference to its blossom or cultivated for its floral beauty or in botany it is that part of a seed plant comprising the reproductive organs and their envelopes if any, especially when such envelopes are more or less conspicuous in form and colour. Flowers have an aesthetic value to man; for instance the lily is a symbol of purity and innocence. The beauty and charm of the blooms can be enjoyed throughout the year. They can be planted in the midst of shrubs to continue their beauty in gardens and can also be grown as pot plants.

The most important varieties of cut flower being exported are roses, chrysanthemums and carnations. The Kenyan local market is very open with the prices determined by supply and demand factors. Some cut flowers are sold locally in main urban centers by street vendors and floricultural shops in high/medium class shopping centers. The distribution of the produce is either sold through the wholesaler markets, directly to the retailers or passes through the hands of middlemen. Hawkers and street vendors are playing a considerable role in the distribution and sale of cutflower.

There is evidence that the trend towards a greater variety of flowers is increasing demand for less traditional varieties of both temperate and tropical flowers (UN, 2001), According to Patrick (2003), florists and clients are in search of new flowers to create new designs that appeal to clients who are looking for novelty. Other than the agronomic traits, novelty is a very important aspect in successful marketing of new varieties such as ornamental millet *(Pennisetum glaucum)*, ornamental pepper' *(Capsicum annuum)* 'Black pepper'*(Piper nigrum),* and *Quinoa(Chenopodium quinoa Willda)*, since this cut flowers are essentially a fashion industry (Chandler, 1996). Flexibility of the products is thus important as is innovation, productivity and quality. Some new varieties fetch prices, which can be up to 7 times higher than those of traditional flowers (ILO, 2000).

To satisfy the demand for new and different flowers, many minor flowering bulbous ornamental plants are being forced either in green houses or outdoors to produce cut flowers (Armitage, 1992). These minor and bulbous flowers are also referred to as specialty cut flowers and are used mainly on special occasions. Nature has produced a tremendous number of bulbous flower genera that provide material for a wide range of aesthetic uses (DeHertogh, 1996). There are many bulbous flowers that are not known or domesticated and hence they fit the class of a different flower. Kenyan flower growers need new introductions of bulbous ornamental plants, such as Oriental lily, Easter lily (*Lilium longiflorum*), Asiatic lily *(Lilium* spp*),* Freesias (*Freesia corymbosia*) and *Ranunculus abortivus* since they have a potential demand in the export market. Lily is one of the main bulb crops in the world. About 75% of the total world production takes place in the Netherlands.

Lily production in Kenya is done by large scale farmers who import pre-chilled bulbs that are ready for planting. Its cultivation amongst small-medium scale farmers is limited by the high cost of production i.e. capital investment in electricity and refrigeration equipment. The low temperature treatment is done for 6-8 weeks before planting. Alternatives need to be sought to reduce the pre-planting treatment duration.

Kenya Agricultural Research Institute has also introduced Easter lilies to small scale farmers in Kiambu and Thika districts. Commonly grown cultivars of Easter lily in Kenya include 'Snow Queen', 'Magie Blanche' and 'White Fox'. Asiatic lilies cultivars include 'PratoBrunnello', 'Romano', 'Fabriano', 'Connecticut King' and 'Adelina'; the Oriental lilies are: 'Star Gazer', Tiber, 'Aktiva', and 'Siberia'. Since introduction in Kenya in 2000 the production has been declining because of lack of understanding of the proper pre-planting treatments especially the requirement for chilling to break dormancy in bulbs.

The lily requires a number of growing seasons before attaining the optimal sized bulbs that can produce high-quality flowers. Lilies need a certain period of cold for rapid shoot emergence and flower formation. According to Ohkawa, (1979), cold period can be partially substituted by the application of gibberellins and benzyladenine. However, due to lack of appropriate storage facilities and technical knowhow and the high cost of construction of cold stores, growers are unable to produce uniform and quality flowers year after year. This study therefore aimed to investigate the influence of plant growth regulators as an alternative treatment to chilling for breaking bulb dormancy and also elucidate their effects on the growth, flowering and multiplication of Oriental lily.

1.2 Statement of the Problem

Floriculture is the main export sub sector of Kenya's horticultural industry and with the diversification of flower species it continues to attract more producers. Rose production dominated the industry for a long time, but presently there is a growing interest in bulb crops and Oriental lilies in particular. Even though lilies earn high profits, their cost of production is prohibitive, especially to small scale growers, owing to the requirement for chilling treatments of the planting materials. Lily bulbs undergo deep physiological dormancy that requires a pre-plant chilling treatment at 4°C for 8 weeks to overcome the dormancy period. Under commercial

production this treatment can only be achieved under high capital investment in refrigeration equipment that also incurs high electricity costs. It is difficult for small growers who cannot meet these expenses and break the dormancy of bulbs. Thus the problem requires research attention to come up with innovative alternative methods of dormancy mitigation to cut farmers' costs and make lily production more affordable and profitable to small scale growers.

1.3 Objectives

1.3.1 Broad Objective

The overall objective of this study was to contribute to the knowledge on sprouting of Oriental lily (*Lilium* spp.) bulbs through use of plant growth regulators as an alternative treatment to chilling.

1.3.2 Specific Objectives

1. To determine the influence of gibberellic acid and benzyl adenine on sprouting of lily bulbs.
2. To establish the influence of gibberellic acid and benzyl adenine treatments on growth and flowering of lily
3. To determine the influence of gibberellic acid and benzyl adenine on bulblet multiplication in lily
4. To establish the interactive effects of gibberellic acid and benzyl adenine on growth, flowering and multiplication of lily
5. To establish whether the use of plant growth regulators is a cost-effective method in breaking dormancy in Oriental lily bulbs.

1.4 Justification

Lily production has great potential for profitability by small scale growers if the challenge of handling and treatment of planting materials can be mitigated by alternative cheaper methods. The use of growth regulators has been reported to mitigate dormancy and substitute the need for chilling in some plants propagated using underground storage and special structures such as corms and tubers which have some physiological and functional similarities with bulbs. Apart from cost savings on chilling treatments, treatment with growth regulators can save on time and hence accelerate the production schedule of a particular crop. This research was therefore carried out to test the effects of growth regulator gibberellic acid (GA_3) and benzyl adenine (BA)

treatments on dormancy breaking in Oriental lily bulbs. Findings of this study contribute to saving costs for small scale bulb flower producers in Kenya.

1.5 Null Hypotheses

1. Gibberellic acid and benzyl adenine have no influence on sprouting of lily bulbs.
2. Gibberellic acid and benzyl adenine have no influence on growth and flowering of lily.
3. Gibberellic acid and benzyl adenine have no influence on bulblet multiplication of lily.
4. There is no interaction between gibberellic acid and benzyl adenine on growth, flowering and multiplication of lily bulbs.
5. Plant growth regulators are not cost effective in breaking dormancy in Oriental lily bulbs.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botany of Lily

The Lily bulb belongs to the family Liliaceae; order Liliales and the genus is Lilium. Plants belonging to family Liliaceae are also important ornamental or houseplants that are grown for their attractive flowers. According to Liang Song-yun (1995), the IranoTuranian region has many species of this group of plants but the Eastern-Asian region is the centre of diversity of Liliaceae. The author believed that *Lloydia, Erythronium, Fritillaria* and *Lilium* originated from the North temperate region, whilst *Gagea and tulipia* are associated with the Old World temperate region. The West Asia to Himalayas and South West China are rich in Notholirion, whilst Cardiocrinum and Nomocharis are natives of East Asia. Afrozi and Hassan (2008) described the family Liliaceae. In general *Liliaceae* comprises perennial plants that have starchy rhizomes, corms, or bulbs. *Liliaceae* is also made up of about 280 genera and 4,000 species. Leaves of plants from this family are alternate or less often opposite or whorled. The flowers are often showy, bisexual and actinomorphic. The perianth may consist of two whorls of undifferentiated or nearly differentiated petaloid tepals with three distinct members in each whorl. The androecium may consist of six fertile stamens attached to the receptacle. The gynoecium, however, is made up of a single compound pistil of three carpels, a single style commonly with three stigmas, and a superior ovary with three locules, each containing several to numerous axile ovule. (De Hertogh and Le Nard, 1993).

The flowers of *L. longiflorum* (Easter lily) are usually white coloured, the flowers of the Asiatic hybrids are usually orange, red, sometimes yellow and white, while the Oriental flowers have more red, pink and white colors. The Oriental flowers have the strongest fragrance (Wilkins and Dole, 1997). The Oriental hybrids are late flowering, while the Asiatic bulbs have a range of flowering times. According to Rees (1992) lilies are classified based on their broad physiological differences, for instance, Asiatic hybrids, derived from at least seven Asian species, have early flowering characteristics (8-10 weeks), upright flowers, disease resistance and are readily propagated. Common Asiatic hybrid cultivars include Apeldoorn and Connecticut king.

Oriental types have flat recurved flowers and flower in 14-16 weeks. Common cultivars include Star Gazer, Aktiva, and Tiber.

Easter lily has white funnel shaped flowers that face outwards. Lily bulbs vary in structure between species. For example, *Lilium longiflorum* has large globular bulbs up to 10 cm in diameter, *Lilium nepalense* has bulbs with broad thick scales that are purple in colour and about 8-10 cm in diameter while bulbs of *Lilium pardalinum* are scaly rhizomes (Arora *et al.*, 2002). The main organs of an adult lily plant are basal roots, the bulb, which is the underground part of the shoot, and the inflorescence or the flowering shoot that emerges above the ground and carries the flowers. The size and form of these organs vary greatly within the genus. The bulb scales are morphologically specialized storage leaves containing nutrients and water. The apical meristem produces the inflorescence whereas the axillary meristems produce the side bulbs that continue growth after the main shoot perishes (McRae, 1998).

The inflorescence bears the leaves that lay either in whorls or are scattered along the stem. The flowers are borne on top of the stem either individually, in racemes or in umbels. In the genus Lilium, the flowers are generally on a stalk subtended by a bract. Each flower has six perianth segments, in two whorls, making a large, attractive bloom, frequently marked or spotted. The form of the flower varies between species and cultivars, some being pendulous, others erect or horizontal; variation also exists in the extent of recurving of the perianth segments. In *Lilium giganteum* the leaves are large, broad and heart shaped with long petioles. Rees (1992)

Some like *Lilium candidum* have radical leaves which grow in the autumn then die, leaving bases which store reserves. Within these are true scales, which are external to the current season radical leave bases. In the axil of the innermost radical leaf alongside the emergent shoot a new daughter bulb is initiated, in late autumn. Other lilies, such as *L.martagon,* have no radical leaves; in these there are no leaf bases acting as storage organs. Flowers are initiated after shoot emergence and initiation of all the leaf primordial. During the spring the daughter bulb initiates new scales until the mother plant flowers; thereafter it grows until it is as large as the surrounding mother bulb and leaf primordia. Large mother bulbs frequently produce two

daughter bulbs and depending on species, many lilies produce at flowering time, small bulbils in the axils of aerial leaves as well as those underground. Rees (1992)

2.2 Development and Growth of Lily

The phases during the development of the shoot apical meristem include juvenile phase, vegetative adult and the reproductive adult phases (Poethig, 1990). The shoot apical meristem has the ability to flower. During the vegetative adult phase the capacity to flower is however not expressed because specific environmental or developmental signals are required (Taiz *et al.*, 1998). Lily species are divided into epigeal and hypogeal, depending on their emergence. Epigeal seeds germinate immediately after sowing without showing any signs of dormancy. The cotyledon emerges from the seed as the radicle intrudes into the soil. The growth of the small seedling continues, and a small plant is formed without dormancy.

Hypogeal germination is usually controlled by dormancy which breaks only after the seed has been exposed to cold treatment. During germination, the radical grows out of the seed but the cotyledon stays within the endosperm conducting reserve nutrients to the forming primary bulblet. Dormancy is often re-induced after the primary bulblet has formed, thus a cold period is required for further development of the plant. The germination type seems to have evolutionary significance since it is species-specific and therefore genetically inherited. Most of the Eurasian and American species represent the delayed hypogeal type of germination whereas most East Asian species represent the immediate epigeal type.

2.3. Lily bulbs

Oriental hybrids are dormant at harvest because of high levels of the abscisic acid (ABA) and a low respiratory activity (Gude *et al.*, 2000b). After harvest, Oriental hybrids bulbs are kept at 2 to 4 °C for at least 8 weeks prior to greenhouse forcing (Wilkins and Dole, 1997). To enable growth and development, a period of low temperatures after harvest is required to break dormancy and to decrease ABA levels (Gude *et al.* ,2000a).

When the bulbs are to be stored for longer periods, temperatures are further reduced to -1 to -4 °C. In this way the bulbs can be stored year-round (Wilkins and Dole, 1997). When storage is

continued at temperatures just above 0 °C, the shoot continues to grow out of the bulb and becomes sensitive to mechanical damage, which can cause a decrease in flower quality. At freezing temperatures, growth of the shoot is arrested. During storage at frost temperatures, actual freezing of the bulbs does not occur (Miller and Langhans, 1990). Lily bulbs are stored in moist peat, especially when kept at freezing temperatures to prevent dehydration (Hartsema, 1961; Beattie and White, 1993).

2.4 Dormancy

Dormancy has been defined as a period of 'rest' (Linkosalo, 2000). Some bulbs contain many meristems at different stages of activity; hence dormancy may be manifested differently under natural conditions or in tissue culture. After flowering, the bulb undergoes a period of suspended growth .The period of dormancy is important both biologically as well as commercially. Biologically it helps the bulb to survive unfavourable hot and cold temperatures.

Commercially, the dormant period becomes a convenient stage for handling, transportation as well as control over flowering. A bulb, corm or tuber with normal emergent shoot or roots is apparently 'dormant', and therefore protected from the unfavourable environment, but examination of the apical meristem can often reveal unexpected activity; leaf primordia, roots or flowers might be in the process of being initiated, be developing or growing (Rees, 1992). Such activity illustrates the success of the survival strategy during the unfavourable period whilst simultaneously providing for the continuation of developmental processes despite the adverse conditions.

Dormancy has been well characterized in peach (Faye *et al.*,1999). At this physiological state the bulb reserves are not available for growth of the daughter organs. Most lilies develop dormancy to survive winter; their bulbs sprout and grow during spring and summer in the temperate zones. This dormancy is broken by a cold period of 6-8 weeks at approximately 4° C. The time needed for dormancy breaking differs with species and genotype (Beattie *et al.*,1993). Bulbous plants such as Tulips and Hyacinth during dormancy show active organogenesis, including flower bud differentiation, vegetative bud differentiation, and root initiation. However in gladiolus and lilium organogenesis is suspended (DeHertogh and LeNard, 1993).

2.4.1 Importance of dormancy in lily flower production

Most lily species require low, non-freezing temperatures to accelerate shoot emergence and flowering (Roh *et al.* ,1977a; Roh, 1985). The exposure either in natural or in artificial cold treatment, is called vernalization - a natural survival mechanism common to certain fall planted species to tolerate low temperatures during the winter. This is caused by a decrease in the number of primordia that become leaves (Roh *et al.*, 1977a, b; Roh, 1985). The plant response to vernalization is brought about by a combination of the temperature during the vernalization period and the duration. When dormancy has been broken, the shoot primordium, located in the centre of the bulb, elongates and leaves unfold. In small lily bulblets however no stem internodes are formed. The primordium in the centre of such bulbs does not elongate, but splits off one or a few leaves whose petioles swell to form leaf bearing scales later in the trial. The site of dormancy in Oriental lily bulbs is considered to be the daughter scales (Roh *et al.*, 1977).

2.4.2 Factors influencing duration of bulb dormancy

According to Arora *et al.* (2002) dormancy in gladiolus corms is due to the presence of high quantities of the growth regulator ABA in freshly harvested corms. Gladiolus exhibits dormancy in response to hot temperatures and this is more pronounced in corms produced under warm climates; those produced under cool climates express little or no dormancy (Arora *et al.*, 2002). Additionally moisture has been shown to prolong the rest period in gladiolus corms (Denny, 1938). Apte (1962) speculated that the existence of a solid and hard tunic, which probably limits gaseous exchanges, also contributes to a longer period of dormancy in gladiolus cormels. DeHertogh and LeNard (1993) pointed out that most bulbs contain fairly high moisture levels and have dry matter contents of about 30% which probably constitutes one of the major factors that prevents true physiological rest during storage. There are some exceptions e.g. Anemone and Ranunculus, whose bulb dry matter content is as high as 85-88%. Consequently, these bulbs can after proper curing, be stored for more than one year without damage.

Hemberg (1985) studied the effects of exogenous growth regulators on dormancy and changes in exogenous growth hormones in potato tubers. Auxins did not influence dormancy itself but only on sprout growth after dormancy had ended. Gibberellins and cytokinins were generally considered to be growth promoters and were capable of breaking dormancy when used as

external applications. Abscisic acid inhibited bud growth and along with other inhibitors overcame the effects of promoters and disappeared when dormancy was broken.

2.5 Forcing

For the control of flowering, needed for the year-round production of flowers, bulbs are subjected to specific temperature regimes to affect their growth and development. The use of artificial growth conditions for the flowering of bulbs, to simulate those required by nature, is called "forcing" (De Hertogh and Le Nard, 1993). Forcing of flower bulbs starts at harvest during dormancy. The applied temperatures during the phase of enlargement affect the physiological state of the bulb at harvest. However, the temperatures in this period can hardly be controlled since it usually occurs outside on the field.

Elevated temperatures applied directly after harvesting are used to control the time and rate of flower formation (De Hertogh and Le Nard, 1993). Subsequently, low temperatures are applied to differentiate the flower bud and initiate rooting. The applied low temperatures are usually required to break the dormancy of flower bulbs, which is defined as the state of a healthy bulb, characterized by little or no external growth of the shoot and roots. After the requirements to break dormancy are fulfilled, bulbs are planted and transferred to a rooting room (to promote root development) or directly to the greenhouse. Depending on the chosen temperature regime flowering can be promoted, retarded or prevented.

2.6 Plant Growth Regulators

Commercial growers have to meet both aesthetic and practical requirements in order to deliver a high quality plant. Cultural practices used by growers involve manipulating environmental factors such as light, temperature, water, and nutrients. Sound cultural practices are the single most important factor in influencing plant growth and quality (Keever, 1994). However, manipulating the environment is not always possible due to factors such as cost or the presence of mixed crop types in a greenhouse. One alternative method that growers use to manipulate plant quality is by physically altering the shape of a plant by means of staking, pruning, and pinching. This is costly as it is not conducive to computerization and requires manual labor

(Puglisi, 2002). Hence growers may choose to use chemical applications in order to manipulate plant characteristics. These chemicals are called plant growth regulators (PGRs).

Appropriate PGR use improves plant quality and yield. Conversely improper PGR use can delay flowering, damage the growth habit or cause phytotoxicity. Growth regulators are used by growers to aid plant propagation by improving seed germination, improving the rooting of cuttings, and triggering the growth in `plant tissue cultures. Plant growth regulators are also used during production to reduce or increase the growth rate of plants, chemically pinch plants, induce buds to break dormancy, break apical dominance, and to delay senescence of buds, flowers, or leaves.

Plant growth regulators for use in the ornamental plant industry were first available in the 1920s to the 1950s (Agranova, 1997) and consisted primarily of natural or synthetic auxins and gibberellins. Some of the older chemicals are toxic and were replaced by more effective modern compounds such as chlormequat (Cycocel), which was commercially available in the 1960s (Albrecht and Tayama, 1992). This was soon followed by daminozide (B-Nine) and ethephon (Ethrel, Florel). In the 1970's ancymidol (A-Rest) and dikegulac- sodium (Atrimmec) were released. In the 1980's, the triazine type PGRs paclobutrazol (Bonzi) and uniconazole (Sumagic) were released along with flurprimidol (Cutlass) and prohexadione-calcium (Apogee). The 1990s saw the release of cyclanilides (Finish). In the 2000's many new products have become available including generic forms of older chemicals (Dazide, Piccolo, Concise), new bedding plant registrations for existing chemicals (Tiburon, Topflor, Configure) and novel modes of action such as with abscisic acid (s-ABA). PGR sales for all agricultural applications represent 3 to 4% of the total pesticide market and in 2002 totaled $0.9 to $1.2 billion per year (Menendez, 2002). The largest uses of PGRs are for cotton defoliation, fruit and nut ripening, and vegetative growth control of cereals and grass.

2.6.1 Uses of plant growth regulators

Plant growth regulators are chemicals that are applied in low doses, absorbed into the plant through the epidermis, and transported to a site of action. They are then recognized and bound to a receptor which amplifies the signal by activating a secondary messaging system to trigger or inhibit a cellular activity. In this regard PGRs are similar to plant hormones (Puglisi, 2002) and

some PGRs are plant hormones. If the concentration is too low, is mal-absorbed, or if the plant is not able to perceive the chemicals then the PGR application will not have the desired effect. As a result PGR activity depends on environmental factors that influence absorption and on the physiological status of the plant. GA_3 has been found to increase the photosynthetic rate in leaves of plants. It has been suggested that GA_3 treatment could lead to changes in plastid development and chloroplast structure. (Ashraf *et al* 2002).

2.6.1.1 Inhibition of growth

Commercial production of ornamental plants may involve growing in crowded conditions with ideal levels of water, fertilizer, temperature, and light. Consequently the plants grow quickly and have a tendency to stretch. Growers often use growth inhibiting PGRs in combination with an environmental change to slow down the growth of a crop or hold it after it is finished. The growth inhibitors are classified as either the anti-gibberellins or the anti-auxins.

Both restrict stem elongation by reducing the plant hormones that trigger cell expansion, or cell elongation. The gibberellin (GA) biosynthesis inhibitors (anti-gibberellins) are by far the most widely used by ornamental plant growers for growth inhibition. Blocking GA biosynthesis reduces cell elongation. At the whole plant level this causes the plant to grow more slowly or not at all.

2.6.2.2 Branching

Commercial ornamental plant growers regularly need to promote branching in certain crops that form long runners such as verbena, English ivy, and lantana or in stock plants to be used as sources for cuttings. The PGRs that are used for this are known as branching agents. They work by interrupting apical dominance, which triggers lateral buds to grow and fill in the plant. Branching agents may also be used to inhibit upward growth due to the fact that a plant which is expending energy on branching has less energy for upward growth. Apical dominance can be interrupted in several ways. One way is to reduce the internal ratio of auxin to cytokinin by applying external cytokinins. Or to apply a chemical that inhibits auxin production or transport. A third way is to kill the apical meristem which halts auxin production (Bangerth *et al.*,2000).

2.6.2.3 Ethylene promoters

Ethylene promoters cause ethylene to be generated inside the plant, which has many possible effects. Ethylene can cause flower, fruit, and leaf abscission (Pederson *et al.*, 2006), leaf epinasty, fruit-ripening, reduce stem growth, and can break apical dominance (Kwon and Criley, 1991). It can induce flowering in bromeliads such as pineapples. Ethylene generally has the effect of delaying flowering for 2 weeks or more (Styer, 2002). By breaking apical dominance, ethylene is able to stimulate branching, and trigger flower buds to break.

2.6.2.4 Growth and flowering enhancers

Commercial growers may want to promote top growth or root growth in their crops. Some ornamental crops *(Poinsettia, Fuchsia)* are grown as standards which have long un-branched trunks with a well branched plant at the top. Growers use growth enhancers to quickly produce the long stem. In some cases, overdoses of growth inhibitors can be overcome with application of growth enhancers (Runkle, 2006). Plant propagators use growth enhancers to stimulate root growth in stem cuttings, and landscapers use them to stimulate root growth to help landscape plants overcome transplant shock (Arteca, 1982). Some growers use PGRs to promote flowering. The plant growth regulators used for this can increase the number of flower buds (Boyle, 1992), increase the size of flowers (Midcap *et al.,* 1999), promote earlier flowering (Sakai *et al.*1979), delay flower senescence (Paulin and Muloway, 1979), prevent bud blasting (Moe, 1979), alter sex ratios in imperfect flowered plants (Amrutavalli, 1980), and induce parthenocarpy (Hayata *et al.* ,1995).

2.7 Types of Plant Growth Regulators

2.7.1 Cytokinins

Cytokinins were first discovered in 1955 as promoters of cell division in the DNA of Herring sperm. Since that discovery many different compound with cytokinin activity have been discovered, both natural (trans-zeatin; benzyl adenine) and synthetic (diphenylurea). The natural occurring active cytokinins tend to be derived from adenine and have either an aromatic or isoprene derived side chain on the N^6 terminus.

These are plant hormones and along with auxins, is part of the plants' hormonal mechanism of enforcing apical dominance. Cytokinins are formed in the roots and are in balance with auxins

moving downward from the shoot tips. Growers can apply exogenous cytokinins to interrupt the balance in favor of cytokinins, which can result in lateral buds escaping apical dominance and breaking. Most exogenous cytokinins are quickly metabolized in the plant which means that they have little carryover.

Cytokinins do not affect the apical meristems in any long term fashion which means that once the lateral buds start to grow, the plant can return to its prior balance of auxin and cytokinins. Subsequent lateral bud growth is optimal in the presence of auxin and cytokinins which have been studied extensively. In the 1960s there was extensive research into using cytokinins to increase the post-harvest life of vegetables such as celery (Guzman, 1963), and lettuce . Others later applied this knowledge to cut flowers like calla lily (Skutnik *et al.,* 2001), Easter lilies and carnations (Heide and Oydvin, 1969).

These PGRs can delay senescence in flowers and prevent age-induced, stress-induced or cold-induced yellowing of leaves. Cytokinins are utilized in many different ways within plants. In new tissues, they regulate cell growth, cell division; In addition they are involved in regulating the cell division cycle (Halmann, 1990; Davies, 1994). Research indicates that cytokinins also induce cell division on en-vivo plants (McCarthy and Bünemann, 1981) and morphogenesis. In callus and wound tissue they regulate the formation of vascular elements. In mature tissues they stimulate chlorophyll biosynthesis, regulate nutrient partitioning, open stomata and delay senescence.

In flowers cytokinins regulate sex determination and pollination. In seeds and fruits regulate dormancy germination, and delay of senescence controlling the balance of roots and shoots. Cytokinins stimulate shoot formation and vascular differentiation in plants and bud formation in mosses. At high levels, cytokinins inhibit root formation, but they promote it at low concentrations. Thus, cytokinin levels are manipulated in tissue culture to induce root formation (low levels), induce callus growth (medium levels), or induce shoot formation (high levels). There is also some evidence that cytokinins are involved with regulating phyllotaxy in plants (Giulini *et al.* ,2004) and with regulating meristem function (Kurakawa *et al.* ,2007).

Cytokinins are also reported to substitute for manual pinching Richards and Wilkinson,(1984) and can thus save growers money. However, the results are not always ideal. In the case of grafted roses, cytokinins increased branching, but some of the branches occurred at the graft union. When cytokinins are used on stock plants to create more cuttings, they sometimes have the effect of reducing the ability of the cuttings to form roots (Day and Loveys, 1998).

Cytokinins are effective in many cases, but are often not as effective as a mix of cytokinin + gibberellin (Fascination, Fresco, Chrysal BVB), GA-alone, or ethylene blockers such as STS and 1-MCP. Very little work has been done on combinations of 1-MCP and cytokinins, but there is some evidence that they act additively in preventing senescence (Çelikel *et al.*, 2002). Cytokinins prevent senescence primarily by reducing the plants' sensitivity to ethylene (Pech *et al.*, 2004), by blocking ethylene biosynthesis within the plant and by antagonizing the effect of abscisic acid on triggering tissue senescence (Halmann, 1990). High endogenous carbohydrate levels and low light levels negatively regulate cytokinins ability to prevent senescence (Drüge, 2000).

Cytokinins may be involved with the photoperiod response of plants (Grayling and Hanke, 1992; Metzger, 1995). They appear to stimulate flowering in some short day and long day plants, but not in others. Endogenous levels of cytokinins have been observed to increase at the start of short days in the SD *Perilla, Begonia* (Hansen *et al.* ,1988), *Chenopodium* (Ullmann *et al.* 1985), and *Mercurialis* (Chang *et al.*, 1999) as well long day plants such as Sinapis. However, some plants such as Xanthium, sunflower, lupine, and tomato show a drop in endogenous cytokinin levels during floral initiation (Letham, 1994). Transgenic Arabidopsis plants (a Long day plant) that are deficient in cytokinins flower later than normal while those that are enriched in cytokinins flower earlier than normal (Bernier and Perilleux, 2005).

These plant growth regulators promote leaf expansion (Mok, 1994) when applied to individual leaves. They do this by regulating assimilate partitioning, cell division, and cell wall extensibility. Transgenic plants that overproduce cytokinins also have altered photosynthate partitioning and thus reduced growth in newer leaves (Jordi *et al.* ,2000).

Cytokinins can delay tissue senescence. When cytokinins are applied to excised leaves, they stay green (Mok, 1994). In the cut flower industry benzyl adenine has been reported to delay the senescence of carnation petals (Van Staden and Joughin, 1988) and other cut flowers by several days. This senescence delay effect has also been indirectly correlated to increased yield of cereal crops because the plants have more time to fill out the grains (Halmann, 1990) and in grapes (Retamales *et al.,* 1995). Exogenously applied cytokinins appear to reduce respiration rates of leaves of some plants (Franco and Han, 1997) which is one of the contributing factors in its ability to reduce foliar senescence. However, they increase respiration in other plants such as *Helianthus, Phaseolus, Citrullus, and Nicotiana* (Musgrave, 1994)

Reversal of stomata closure caused by ABA can be caused by cytokinin and by high levels of CO_2 (Blackman and Davies, 1985). Under drought stress, cytokinin levels drop which de-inhibits ABA from closing the stomata. Once drought stress is relieved, cytokinin levels rise and reverses the ABA mediated closure of stomata. By themselves, cytokinins do not affect stomata in young leaves (Blackman and Davies, 1985). However, in fully expanded leaves, cytokinins are able to cause stomata to open without the presence of ABA. Thus exogenously applied cytokinins increase transpiration rates of plants (Blackman and Davies, 1985), but the response varies based on the age of the leaf and the plant species. Cytokinins generally, increase the ratio of female flowers to male flowers which has implications for fruit production (Halmann, 1990).

Cytokinins have been shown to regulate the growth of xylem fibers and sieve tube elements in coleus (Aloni *et al.* ,1990). This effect could be useful in accelerating the formation of a graft union and thus improving graft take percentages. Cytokinin levels change throughout the year as the new plant tissues age. The levels are low in dormant plants, rise in the spring when dormancy is released and fall again in the summer as summer dormancy occurs. Finally, there is a drop at the beginning of winter. The levels of cytokinins vary in individuals of the same species and affect the individual's proclivity for sylleptic branching (Cline and Dong-Il, 2002).
Cytokinins seem to be involved with many types of stress resistance mechanisms in plants (Hare *et al.,* 1997) including drought (Chernyad'ev, 2005,), high temperatures (Xu and Huang, 2007), cold (Ranwala and Miller, 2005), herbicides (Durmus and Kadioglu, 2005), and shipping Beach,

(2005;). Thus, exogenous cytokinins could be used to protect plants during stressful production conditions.

Tsukamoto (1972) and Ginzberg (1973) have shown that cytokinins can break dormancy in gladiolus corms. Such treatments have been useful for plant breeders to shorten the growth and development cycle.

Nagar (1995) reported that there was a good correlation between free ABA levels and degree of dormancy in tuberose bulbs. Suttle *et al.*, (1994) noted that the ability of ABA to regulate meristem dormancy in tissues varies with plant organs; its role in dormancy may be more universal than was once generally acknowledged. According to Khan (1975) free ABA alone does not necessarily inhibit growth in dormant seeds and bulbs. In lily bulbs, a threshold level of endogenous ABA is required for the expression of dormancy but an additional unknown factor may also play a major role (Kim *et al.*, 1994).

2.7.2 Gibberellins

Gibberellins are a class of plant growth regulators that affect many aspects of plant development overcome dormancy, increase flower size, flower number, flower uniformity, and to create standards. GA_3 is the first widely available active form of commercial GAs. (Martin 1983) It is an economically important secondary metabolite formed as the end product of the GA pathway (Takahashi *et al* 1986). GA_3 acts as a hormone, regulating several processes of plant development. (Takahashi *et al* 1986). It works with other hormones to promote rapid elongation and division of cells. They may also be used to help overcome anti-GA PGR overdoses. When using GA's to overcome PGR overdoses, it is important to apply very small doses and watch the crops closely for an effect. A gibberellin overdose will result in a spindly unmarketable plant (Runkle, 2006).

Gibberellins are most commonly used on camellias to induce early flowering and increase flower size via a process called 'gibbing' (Midcap *et al.* ,1999). Research on gibberellins has mainly focused on the relationship of endogenous changes to low temperature treatment; the endogenous level of GA is lower in dormant bulbs compared to non dormant bulbs (Kim *et al.*,1999). Thus, gibberellin is used for breaking dormancy. Favourable effects of plant growth regulators on

germination, growth, flowering and multiplication in gladiolus have been reported by Roychoudhury (1985). Barman *et al.* (2004) observed that benzyladenine enhanced sprouting at higher concentrations whereas the reverse trend was observed with gibberellic acid. Wang *et al.*, (2000) made similar observations on seed germination of chrysanthemum and some vegetables. Barman *et al.* (2004) reported 100% sprouting in gladiolus corms that had been soaked for 24 hours in gibberellic acid but Goo *et al.*, (1998) noted that BA was better compared to in terms of sprouting of in vitro produced gladiolus corms. Kirad *et al.*, (2001) also noted that gibberellic acid increased the sprouting of gladiolus corms. Gibberellins were effective in breaking dormancy in potato tubers. Studies have shown that GA_3 promotes growth. (Khan *et al.*, 1998)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental site

Field experiments to study the performance of Oriental lily subjected to different hormonal treatments of Gibberellic acid and Benzyl adenine were carried out at James Finlay Lemotit farm, at Kericho for two trials. The site was selected because Lemotit farm is located in the highlands and the climatic conditions within the area is conducive for growing of lilies. The first trial was conducted from September 2009- January 2010, which was characterized by high temperatures while the second trial was conducted from January 2010 to April 2010 which was characterized by low temperatures and overcast weather. The farm is located at approximately latitude 0°22°"South and longitude 35°18°"East. Annual rainfall in the last four years had been an average of 1386 mm/year (Finlay flowers weather data 2009). The average maximum temperature was 24°C and the minimum temperature was 9°C, with relative humidity of 85%. The experiment was conducted in tunnels with shade net covering. The land was ploughed deeply and the soil clods harrowed to a fine tilth. Plots were demarcated and made into raised beds (15 cm) measuring 1 m wide. Tea compost manure was mixed with the soil at a ratio of 1:1. and mono ammonium phosphate fertilizer uniformly applied at the rate of 4.4 g/m^2; the beds were watered thoroughly before planting.

3.2 Planting

Lily bulbs were planted manually after 12 days of PGR treatment. The bulbs were spaced at 20 cm inter row and 20 cm intra row . All the other cultural practices including pest and disease control, irrigation and support were applied uniformly in all the three blocks. Irrigation was done using a gravity operated drip system installed in the beds.

Three drip lines (0.36 cm wall thickness, 0.25 cm emitter spacing) were placed in between the rows in the beds. Nylon net of eye spacing 10 cm X 10 cm, 1 m wide was placed on the beds to train the plants during their growth. The net support ran parallel to the beds and was supported by poles 2 m high and 1 m apart. The net support was lifted gradually after the plants had sprouted to evade the plants from bending or deformation. Harvesting began as soon as the first bud had attained horticultural maturity when the bud had opened at the apex and had attained an

increase in size and the colour of the bud had turned pink for the variety Tiber. Secateurs were used to cut the stem with the mature buds 10 cm from the crown.

3.3 Experimental treatments and design

The treatments used were plant hormones Gibberellic acid and Benzyl adenine, in concentrations (singly, and in combination), no hormonal treatment and chilled Oriental bulbs. Bulbs of the variety Tiber were used. Oriental lily bulbs of size 5-8 cm were soaked for 24 hours in buckets containing different concentrations levels of GA_3 and BA (namely, 0, 25, 50,100, and 150 mg/l) and their combinations, respectively. Subsequently; the bulbs were kept under shade in a well ventilated store for 12 days before planting.

The treatments were laid out in an unbalanced factorial in a Complete Randomized Block Design (RCBD). The field layout was row plots of 26 treatments - 25 treatment combinations of GA_3 and BA plus one treatment of chilled bulbs. Each treatment had 10 bulbs replicated three times. Each replicate of the experiment contained all GA_3: BA treatment combinations and 10 chilled treatment bulbs as a positive control. The untreated bulbs served as the negative control.

3.3.1 Field Layout

The treatments were laid out randomly in three blocks. The block randomization scheme is shown below:

Block 1

T 11	T 7	T 14	T 6	T 15	T 8	T 25	T 20	T 16	T 4	T 22	T 3	T 24	T 9	T 21	T 5	T 12	T 10	T 26	T 2	T 19	T 23	T 18	T 13	T 17

Block 2

T 14	T 7	T 21	T 6	T 25	T 8	T 23	T 2	T 17	T 4	T 11	T 3	T 15	T 9	T 22	T 5	T 24	T 10	T 19	T 16	T 13	T 1	T 12	T 26	T 18

Block 3

T 22	T 7	T 14	T 6	T 15	T 18	T 11	T 2	T 25	T 4	T 17	T 3	T 23	T 9	T 26	T 5	T 20	T 10	T 21	T 1	T 24	T 16	T 13	T 8	T 19

Key

T is Treatment

Numericals are treatment numbers

3.4 Parameters studied

The parameter studied were percent sprouting, number of days to 50% bulb emergence, days to opening of first leaf whorl, leaf number, stem length, number of florets, rachis length , number of days to flowering, bulb weight and number of bulblets formed per mother bulb.

3.4 1 Emergence

The number of visible sprouts after emergence was recorded in each plot. This was used to calculate the percentage of bulbs that had sprouted. This is important to growers because a high percent sprout indicates that the breaking of dormancy was effective.

$$\text{Percentage sprout} = \frac{\text{No. of bulbs sprouted} \times 100}{\text{No of bulbs planted}}$$

In addition the number of days taken to 50% bulb emergence per plot was determined by counting the number of days from planting till when there was 50% bulb emergence in each plot.

3.4.2 Growth

The number of days from planting to the opening of the first leaf whorl was noted in eight randomly selected plants in every plot. The number of leaves, and stem length were used to gauge the vegetative plant response to the gibberellic acid and benzyl adenine treatments.

3.4.3 Flowering

The numbers of florets formed were counted and the lengths (cm) of the flower buds were determined. It was necessary to determine if the two plant hormones had any influence on the number of days taken to harvest or flowering. Harvesting began as soon as the first bud had shown a change in colour and opened at the apex. All buds harvested were free from diseases and pests and fell within the grading specifications.

3.4.4 Multiplication

Lifting of lily bulbs was done after harvesting. At harvest the stems were cut 10 cm above the ground so that the remaining part of the stem would continue supporting the growth of the bulb before lifting. The bulbs were examined for bulblet formation and those with bulblets were recorded and the number of bulblets noted by counting. The bulb weight was determined by weighing the bulbs on an electric weighing balance.

3.5 Data Analysis

Data collected was analyzed using Analysis of Variance (ANOVA) under the General linear model (GLM) procedure (SAS, 1998) at $P \leq 0.05$ to determine treatment effects and interaction. Using the following statistical model

$$Y_{ijk} = \mu + R_i + G_j + L_k + (GL)_{jk} + \varepsilon_{ijk}$$

Where; Y_{ijk} = Plant response,

μ = Constant,

R_i = Effect due to i^{th} block,

G_j = Effect of Gibberellic acid,

L_k = Effect of Benzyl adenine effect,

$(GL)_{jk}$ = Interactive effect of the j^{th} level of Gibberellic acid and k^{th} level of Benzyl adenine,

e_{ijk} = Error

3.6 Economic Analysis

Cost benefit analysis [(yield x price)-other variable costs – cost of factor (benzyl adenine and /or gibberellic acid) (Abbot and Makeham, 1979] was done to determine the relative profitability of using benzyl adenine or gibberellic acid. The Oriental lily yields used in the analysis were the yields of experimental plots. However, because they are typically at the high end of yields realized by growers, the results were subjected to sensitivity analysis for expected, optimistic and pessimistic yield scenarios. Experimental yields are considered optimistic. A more realistic or expected yield level would be 80% of the optimistic yield while a pessimistic or poor yield would be 60% of the optimistic. Thus to arrive at the expected and pessimistic yields, the yields from the experimental plots were multiplied by 0.8 and 0.6 respectively for the sensitive analysis. Kenyan small scale growers' average yields are considerably lower than these but reflect a prevalence of less intensively managed systems.

Gross returns were calculated by matching the actual total yield with the appropriate average lily market price for each trial. The prices used in this analysis were the seasonal averages used at Finlay's. These prices were Ksh. 60 and Ksh. 80 in trials 1 and 2 respectively. Production costs for the Oriental lily were derived from bulb crop budgets for the James Finlay Lemotit farm. Field operations and amount of chemical inputs reflect actual operations at the experimental site. The general agricultural costs did not vary between trials hence did not require adjustments (Table 11). However, harvest and marketing costs changed with varying yield levels.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Effects of BA and GA_3 on Sprout Emergence (%)

The effect of different levels of the two plant growth regulators (Benzyl adenine and Gibberellic acid) on sprout emergence was statistically significant (Table 1). Results in trials 1 indicated significant effects of gibberellic acid and benzyl adenine on dormancy breaking in Oriental lily bulbs. The highest percentage of sprout emergence was observed when bulbs were treated with 50 mg/l of benzyl adenine (84.67%) as well as with 50 mg/l gibberellic acid (82.0%). These were not significantly different from the positive control treatment of chilled bulbs which attained 100% sprouting. There was a general increase in sprout emergence as the GA_3 concentrations were increased from 0 mg/l to 50 mg/l. This result is in agreement with that of (Langens *et al.* 1997) whereby Gibberellic acid at the rate of 3mg/l was found to be most favorable for breaking dormancy in *Lilium speciosum cv. Rubrum.* Kirad *et al.* (2001) also noted that gibberellic acid increased the sprouting of gladiolus corms.

GA_3 concentrations above 50 mg/l caused a decline in sprout emergence. These findings are similar to those made by Wanjao (1981) on Liatris where GA_3 replaced cold treatment in breaking dormancy.

BA had similar effects in trial 1 where a general stimulation of sprouting was observed with there being no significance difference between 25,50 and 100mg/l. In trial 2, all BA treatments were significantly higher than the negative control. In all treatments with BA, 92% sprout emergence was observed compared to the negative control where only 58% sprouting was recorded. Barman *et al.* (2004) reported 100% sprouting in gladiolus corms that had been soaked for 24 hours in gibberellic acid but Goo *et al.* (1998) noted that BA was better compared to in terms of sprouting of in vitro produced gladiolus corms.

The present results indicate that low concentrations of plant growth regulators promote high sprout emergence therefore breaking dormancy. Gibberellic acid and Benzyl adenine play an important role in cell elongation and cell division. Hence BA and GA_3, being activators of cell division and elongation respectively, played a part in promoting meristematic activity (cell

division and elongation) causing improved sprout emergence where the treatments were applied. Combined treatments of various levels of BA and GA_3 produced significantly different effects on sprouting with the highest sprouting percentage achieved with a combination of 50mg/l BA and 100mg/l GA_3. Similar results were obtained by Kiyoshi (1979) on *Lilium speciosum* where high percentage sprouting was observed with a combination of GA_3 and BA.

In both trials there were no significant effects of combining BA and GA_3 on the percent sprouting. (See appendix 1).

Table 1: Percentage sprout emergence of Oriental lily bulbs under different BA and GA_3 concentration treatments.

Growth regulator treatments (mg/l)	Trial 1		Trial 2	
	BA	GA_3	BA	GA_3
0 (negative control)	59.33c	57.33c	58.33b	66.33c
25	58.00c	76.00ab	92.00a	86.00b
50	84.67a	82.00a	92.00a	92.00ba
100	81.33ab	73.33ab	92.00a	90.00ba
150	69.33bc	64.00bc	92.67a	92.67ba
Chilled bulbs (Positive control)	100.00a	100.00a	100.00a	100.00a

Means followed by the same letter in a column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT)

Plate 1: Sprouting in Oriental lily bulb

4.2 Days to 50% Sprout Emergence

The rate of sprout emergence in the bulbs was measured by determining the number of days to 50% sprout emergence. In trial 1 the time to 50% sprout emergence was significantly influenced by bulb treatment with BA and GA (Table 2). Means of 20, 20, 17, 13 and 21 days to 50% sprout emergence, respectively, were recorded when bulbs were treated with 0, 25, 50, 100, and 150 mg/l of BA. 100 mg/l of BA had the least number of days(13) and was significantly different from the control and the other concentration levels. The results also indicated a significant reduction of sprouting time when BA was used at 50 and 100 mg/l, respectively. In trial 2, a marked improvement in time to 50% sprout emergence was observed. All the BA treatments (25,50,100 and 150 mg/l) applied were not significantly different nonetheless recorded less than 10 days to 50% sprout emergence which was significantly different from the two controls.

The effect of GA_3 levels on the time to 50% emergence followed a similar pattern to those of BA. Specifically, in both trials, GA_3 significantly reduced the time to 50% emergence compared to the controls. Notably, in trial 1, 100 mg/l of GA_3 recorded 20 days to 50% emergence in comparison to the other treatments (25, 50 and 150 mg/l) which recorded 17,16 and 15 days respectively. Studies done earlier indicate that exogenous applications of GA_3 caused a sudden increase in endogenous gibberellin concentrations and quickly lead to visible sprouting (Sonnewald 2001). In trial 2 all GA_3 treatments plus the controls were not significantly diiferent. When the two PGRs were combined at rates of 100 mg/l BA and 50 mg/l GA_3, it took a mean of 9 days to attain 50% emergence. This result could be ascribed to the effect of GA_3 in facilitating movement of cytokinins to newly formed buds leading to increased cell division and accelerated bud break. (Sonnewald 2001).

It has been shown that the effects of GA_3 also correlate with increased cell division. Taiz and Zeiger (2002) suggested that the newly produced cells may act as sinks for carbohydrates and that invertase provokes starch breakdown resulting in sugars being transferred to the sprouts, resulting in growth. Similar reactions may be occurring in the present study where bulbs were treated with PGRs leading to accelerated growth responses.

Table 2: Number of days to 50% sprout emergence of Oriental lily bulbs under different

Growth regulator treatments (mg/l)	Trial 1		Trial 2	
	BA	GA_3	BA	GA_3
0(negative control)	20.00ab	23.20a	12.20a	10.27b
25	20.13ab	17.60bc	9.67b	9.73b
50	17.20b	16.20c	9.13b	9.80b
100	13.67c	20.0ab	9.80b	10.60b
150	21.07a	15.07c	9.73b	10.13b
Chilling(Positive control)	20.00ab	23.20a	12.20a	10.27b

BA and GA_3 concentration treatments.

Means followed by the same letter in a column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT)

4.3 Effect of benzyl adenine and gibberellic acid on the growth of Oriental lily

4.3.1 Number of days to opening of the first leaf whorl

Different concentrations of BA had no significant influence on the opening of the first leaf whorls in trial 1 (Table 3). GA_3 at 50 mg/l significantly reduced the number of days to opening of the first leaf whorl. Combinations of BA and GA_3 produced significant interactive effects in both trials. In trial 1, bulbs that were treated with 100 mg/l GA_3 only took the shortest time (25 days) while those treated with BA 150 mg/l alone took the longest time of 38 days. A trend of a longer time to opening of the leaf whorl was observed in the interaction between 0 mg/l GA_3 and increasing BA concentrations. In trial 2, interaction of 50 mg/l BA and GA_3 at 100 mg/l significantly influenced the number of days to first leaf whorl opening (24 days).

The opening of the first leaf whorl is dependent on the mobilization of sugars. When the period of opening is prolonged it shows that there is insufficient availability of sugars at planting. Only after sometime in the soil during which starch breakdown will continue, sufficient carbohydrates are available for the opening of the leaf whorl. Therefore it is important to have carbohydrate reserves which can be rapidly mobilized to sustain the subsequent leaf development.

Table 3: Number of days to opening of the first leaf whorl of Oriental lily bulbs under different BA and GA_3 concentration treatments.

Growth regulator treatments (mg/l)	Trial 1		Trial 2	
	BA	GA_3	BA	GA_3
0 (negative control)	31.27a	32.67 abc	25.00bc	28.80a
25	31.07a	31.07 bc	25.33bac	24.40b
50	32.27a	30.27c	25.73ba	24.27b
100	31.73a	34.00 ab	24.13c	24.53b
150	33.73a	34.93a	26.80a	25.00b
Chilling (Positive control)	31.00a	31.00a	22.67c	22.67c

Means followed by the same letter in a column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT)

4.3.2 Number of leaves

There was a significant effect ($P < 0.05$) of PGRs on number of leaves produced on the stalks in both trials 1 and 2. In trial 1, treatment with 100 mg/l BA recorded the highest number (39) of leaves whilst in trial 2 the highest leaf count of (44) was observed with BA 25 mg/l which was statistically similar to the chilled bulbs (positive control) (Table 4). This result could be ascribed to the positive effect of BA on the leaf growth resulting in more plastids and chlorophyll leading to an increase in photosynthesis and carbohydrate formation.

Studies indicate that spraying plants with cytokinin compounds such as kinetin and BA promote the opening of stomata on plant leaves, thus enhancing gas (CO_2) exchange, increasing photosynthesis and consequently carbohydrate accumulation in the leaves of treated plants (Stuart, 1952). Cytokinins have been shown to enhance development of etioplasts into chloroplasts and to increase the rate of chlorophyll formation (Salisbury and Ross, 1990). These known cytokinin effects possibly resulted in the higher chlorophyll content resulting in many leaves being formed. The phytohormones activate source function of leaves, through the stimulation of leaf expansion, increase of photosynthesis, changing a balance between transportable and storage forms of photo assimilates (increasing sucrose and reducing starch synthesis (Miller and Langhans 1990). In accordance with the results obtained, BA which is a cytokinin can be used to stimulate leaf growth. These findings are in agreement with studies done by (Ron'zhina, 2007))whereby cytokinins were found to stimulate growth, sink activity of

tubes, and incorporate soluble organic substances into insoluble polymeric compounds such as starch, structural polysaccharides, and proteins.

In both trials, there was no significant difference between different concentrations of GA_3 however 50 mg/l promoted the highest mean number of leaves, 39 and 41, respectively, which were statistically similar to the chilled bulbs (42). Maruya and Nagada (2002) reported that application of GA_3 increased the number of leaves in gladiolus, while Shradha *et al.* (2002) reported similar observations in tuberose. Gibberellic acid is known to improve the photosynthetic efficiency of plants through its influence on photosynthetic enzymes, leaf-area index, light interception and enhanced use efficiency of nutrients (Khan *et al.*, 2007). Therefore with regard to the results obtained in this study GA_3 other than being an alternative to chilling treatment it also influences leaf growth which is important for photosynthetic activity and overall growth of the Oriental lily plant.

There was a trend observed in both trials that as GA_3 concentrations increased from 50 mg/l to 150 mg/l the number of leaves reduced however there was no significant difference between the different concentrations (Table 4). Pranav *et al.*, (2005) reported that significant reduction in number of leaves per plant was noticed with increasing levels of GA_3. When gibberellins are applied, they move to the shoot apex causing increased development of young leaves, thereby enhancing photosynthetic rates and growth of the whole plant.

In trial 2, there was no significant difference between the GA_3 concentration levels and the control. Combined effects of 50 mg/l GA_3 and 0 mg/l BA significantly increased the leaf count resulting in 50 leaves being developed.. The carbohydrates mobilized during dormancy breaking serve as building blocks and energy source for the development of the leaves and the photosynthetic apparatus after planting. Also in seeds, mobilization of reserves is needed to sustain growth post germination Bewley (1997).

A low leaf count was observed in the combined effects of BA and GA_3. Similar findings were made on anemone when tubers were soaked in benzyladenine and gibberellic acid this reduced the number of leaves formed by the tubers to one-third or one-fourth (Janowaska *et al.*, 2009).

In *Lilium* it has been shown that the flower represents the strongest sink for photo assimilates during its development, 85% of the photo assimilates synthesized in the leaf goes into the development of flowers. Therefore leaves are essential in determining flower development.

It has been reported that during low temperature treatment carbohydrate reserves are mobilized by hydrolysis of starch which stimulates the development of the leaves. Hence presence of soluble sugars such as sucrose which is the most commonly translocated carbohydrate (Taiz and Zeiger 1998) , Starch breakdown at low temperature is a phenomenon known as 'low temperature sweetening' (Sowokinos 1990) and also occurs in lily in nature.

Table 4: Number of leaves formed on Oriental lily bulbs under different BA and GA_3 concentration treatments.

Growth regulator	Trial 1		Trial 2	
treatments (mg/l)	BA	GA_3	BA	GA_3
0 (negative control)	35c	35c	40bc	38a
25	36bc	36bc	44a	41a
50	35c	39ab	40bc	41a
100	39a	36bc	37c	40a
150	38ab	36bc	39bc	40a
Chilling(Positive control)	42a	42a	41a	41a

Means followed by the same letter in a column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test (DMRT)

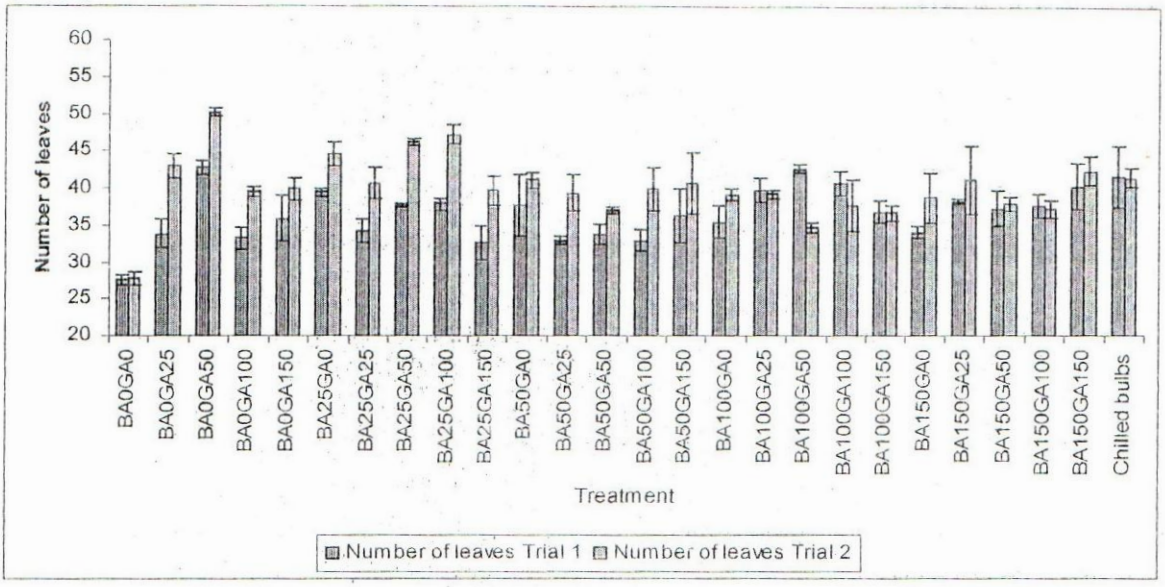

Figure 1: Influence of various combination of benzyl adenine (BA) and gibberellic acid (GA_3) on the number of leaves of Oriental lily bulbs *(Lilium spp)* evaluated in 2009/2010.

(1=0,0; 2=0,25; 3=0,50 ;4=0,100 ;5=0,150; 6=25, 0; 7= 25, 25; 8=25, 50; 9=25,100; 10= 25,150; 11=50, 0; 12=50, 25; 13=50, 50; 14=50,100; 15=, 50,150; 16=100, 0; 17=100, 25; 18=100, 50; 19=100,100; 20=100,150; 21=150, 0; 22=150, 25; 23=150, 50; 24=150,100; 25=150,150; mg/l; 26= chilled bulbs)

4.3.3 Stem length

There was a significant effect of BA and GA_3 on the stem length of Oriental lily in trial 1 (Table 5). The longest stems of 52.01 cm were obtained with 100 mg/l of BA (Table 5). A trend where stem length increased from 41.27 to 52.01 cm was noted as the concentration of BA was increased from 25 mg/l to 100 mg/l.. However, at 150 mg/l, the stem length decreased. This parameter was most affected by hormones. This was observed under high concentration levels of BA and GA_3.

In trial 1 gibberellic acid had a significant effect on the stem length resulting in longer stems than those observed with the BA treatments. This can be attributed to the action of GA_3 in inducing internode elongation. In both trials GA_3 at 50 mg/l promoted the stem length (48.38cm) which was significantly different from the control. A trend was observed that as the concentration of GA_3 increased from 50 mg/l to 150 mg/l the stem length decreased from 48.38cm to 39.01 cm. However 50 and 100 mg/l were not significantly different.

The two PGRs interactive effects significantly influenced the stem length in both trials. In trial 1, the combination of 150 mg/l BA and 50 mg/l GA_3 produced the longest stem length of 63.70 cm but as the concentration of GA_3 increased to 150 mg/l the stem length reduced to 26.9 cm. On average 100 mg/l BA combined with 25 mg/l GA_3 produced stem lengths of 61.90 cm in trial 1. These observations concur with those reported other studies where GA_3 has been found to increase in cell elongation resulting in longer plants (Kaweeta 2003). According to Stephen *et al.*, (2005) GA_3 promotes cell elongation by induction of enzymes that promote cell wall loosening and expansion.

BA at 150 mg/l with 50 mg/l GA_3 resulted in average stem length of 63.70 cm in trial 1 and 52.8 cm in trial 2. Combinations of highest levels of the PGRs (150 mg/l BA and 150 mg/l GA3) considerably reduced stem lengths (26. 90 cm). In trial 2 benzyl adenine 25 mg/l gave the highest stem length of 54.15 cm which was significantly different from 50 mg/l, 100 mg/l, 150 mg/l and chilled bulbs. Gibberellic acid 50 mg/l recorded longest stems of 52.57 cm which were not significantly different from the controls. In the present study the results could be due to the fact that GA's promote stem elongation through stimulation of both cell elongation and cell division

Plate 2: Stems of Oriental lily bulbs

Table 5: Stem length on Oriental lily bulbs under different BA and GA_3 concentration treatments.

Growth regulator treatments (mg/l)	Trial 1		Trial 2	
	BA	GA_3	BA	GA_3
0(negative control)	40.34c	40.91 cd	51.01 ba	48.34b
25	41.27c	46.46 cb	54.15a	51.21 ba
50	43.27c	48.38b	50.07b	52.57a
100	52.01b	45.41 cb	49.70b	52.29a
150	43.29c	39.01d	50.35b	50.87 ba
Chilling (Positive control)	69.07a	69.07a	48.43b	48.43b

Means followed by the same letter are not significantly different at $P \leq 0.05$, according to Duncan's Multiple Range Test.

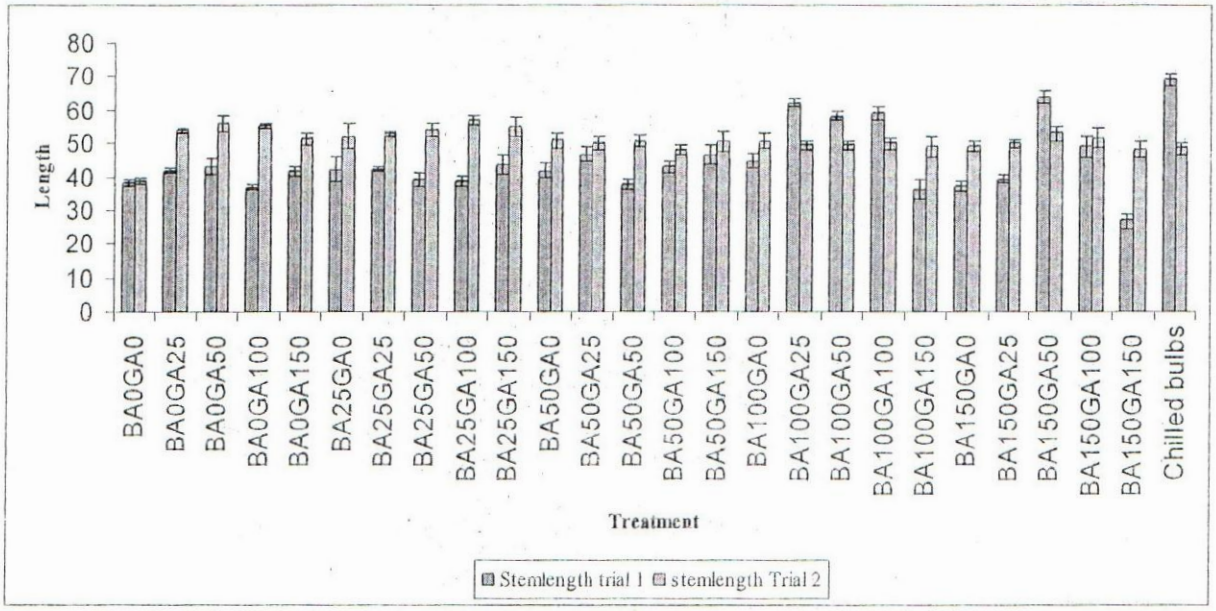

Figure 2. Influence of various combination of benzyl adenine (BA) and gibberellic acid (GA_3) on the stem length of Oriental lily bulbs *(Lilium spp)*.

[(GA3) (1=0,0; 2=0,25; 3=0,50 ;4=0,100 ;5=0,150; 6=25, 0; 7= 25, 25; 8=25, 50; 9=25,100; 10= 25,150; 11=50, 0; 12=50, 25; 13=50, 50; 14=50,100; 15=, 50,150; 16=100, 0; 17=100, 25; 18=100, 50; 19=100,100; 20=100,150; 21=150, 0; 22=150, 25; 23=150, 50; 24=150,100; 25=150,150; mg/l; 26= chilled bulbs)]

4.4 Effect of benzyl adenine and gibberellic acid on the flowering of Oriental lily bulbs

4.4.1 Number of florets.

Results showed significant effects of BA and GA_3 on the number of florets produced in both trials. For the single treatments, BA at 100 and 150 mg/l produced means of 4.13 and 3.00 florets per plant, respectively, in trial 1 (Table 6) which was statistically similar to the chilled bulbs. On the other hand GA_3 at 150 mg/l produced the highest number of florets (3.87); this was also not significantly different from the number of florets produced in chilled bulbs. These findings are similar to those of Janowaska *et al.*, (2009) in anemone tubers soaked in 100 and 150 mg/l GA_3 before planting. A general trend of increasing numbers of florets with increasing GA_3 concentrations upto 100 mg/l was also evident in both trials however there was no significant difference between50 and 150mg/l. This indicates an advantage in terms of GA_3 treatment in promotion of more florets in the spikes of Oriental lilies. According to Corr *et al,* (1987), GA_3 causes an increase in the available substrates at the time of floral initiation leading to profuse flowering. The number of florets formed is an important attribute for growers since the higher the number of florets per stem, the higher the premium fetched. Thus GA_3 may be used to improve floret development in Oriental lilies.

In the interaction in trial 1, BA 150 mg/l promoted the highest number of florets 8.88 . In trial 2, $GA_3$100 mg/l promoted high number of florets while no PGRS interaction treatment resulted in 2.89 florets. 100 mg/l GA_3 with 0 mg/l BA in trial 2 resulted in 4.67 florets which was not significantly different from the chilled bulbs (positive control). This result could be attributed to the positive role of cytokinin in the regulation of inflorescence meristem activity and size (Werner and Schmulling 2009; Kiba and Sakakibara 2010)..

Table 6: Number of florets of Oriental lily bulbs under different BA and GA_3 concentration treatments.

Growth regulator treatments (mg/l)	Trial 1		Trial 2	
	BA	GA_3	BA	GA_3
0 (negative control)	0.00c	0.00c	3.99b	3.50c
25	0.53cb	1.20 cb	4.00b	3.52c
50	1.53b	1.73b	3.89b	3.77bc
100	4.13a	2.40b	3.67cb	4.18ba
150	3.00a	3.87a	3.33c	3.91bc
Chilling (Positive control)	4.00a	4.00a	4.62a	4.62a

Means followed by the same letter are not significantly different at $P \leq 0.05$, according to Duncan's Multiple Range Test.

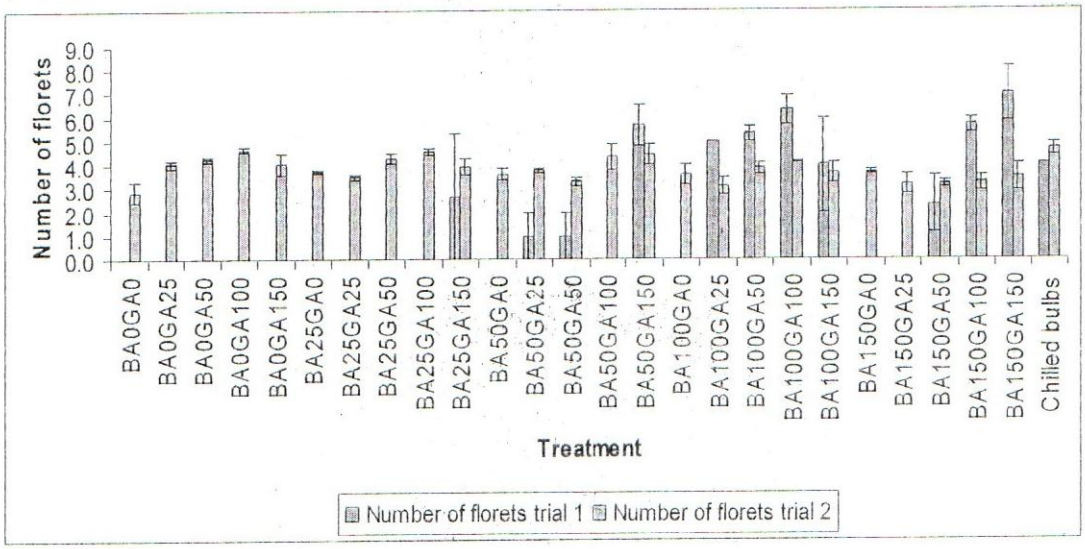

Figure 3: Influence of various combination of benzyl adenine (BA) and gibberellic acid (GA_3)on the number of florets.

(1=0,0; 2=0,25; 3=0,50 ;4=0,100 ;5=0,150; 6=25, 0; 7= 25, 25; 8=25, 50; 9=25,100; 10= 25,150; 11=50, 0; 12=50, 25; 13=50, 50; 14=50,100; 15=, 50,150; 16=100, 0; 17=100, 25; 18=100, 50; 19=100,100; 20=100,150; 21=150, 0; 22=150, 25; 23=150, 50; 24=150,100; 25=150,150; mg/l; 26= chilled bulbs)]

4.4.2 Length of rachis

There was a significant effect ($P<0.05$) of gibberellic acid in both the trials on the rachis length of Oriental lily bulbs (Table 7). In trial 1, the longest mean rachis length of 9.46 cm was observed when bulbs were treated with 25 mg/l GA_3 as well as with 50 mg/l BA (9.19 cm).

These were not significantly different from the positive control treatment of chilled bulbs. In the present study, long rachis lengths were attributed to the synergistic activity of GA_3 and BA which promoted cell division as well as elongation. A trend was observed as the level of BA and GA_3 increased from 25 mg/l to 150 mg/l; decrease in rachis length in trial 1.

Benzyl adenine had similar effects in trial 1, where an increase in rachis length was observed with a maximum at 50 mg/l followed by a decline beyond this concentration. In trial 2 Gibberellic acid 100 mg/l resulted in long stalks; 12.07 cm length was the highest compared to the chilling control 11.10 cm (Table 7). This is consistent with previous reports on GA_3 treated plants which produced an increase in rachis length (Padaganur *et al.*, 2005) The effect of interaction on rachis length was significant in trial 1. The effect of interaction on rachis length was significant in trial 1 only. In trial 2, BA 100 mg/l combined with GA_3 0 mg/l promoted the longest rachis of 10.47 cm, whereas a short rachis length of 2.93 cm was observed when BA150 mg/l interacted with GA_3 150 mg/l. This decrease in rachis length could be attributed to high concentrations of the PGRs.

Devadanam *et al.*, (2007) reported significant increase in rachis length with GA_3 in tuberose. The increased rachis length might be due to rapid internode elongation as a result of increased in cell division and cell elongation in intercalary meristem.

This is consistent with previous reports on GA_3 treated plants which produced an increase in rachis length, (Padaganur *et al.*, 2005) .

Table 7: Rachis length of Oriental lily bulbs under different BA and GA_3 concentration treatments.

Growth regulator treatments (mg/l)	Trial 1		Trial 2	
	BA	GA_3	BA	GA_3
0 (negative control)	8.80a	8.79a	10.63a	8.69c
25	8.76a	9.46a	10.46a	10.43b
50	9.19a	8.89ba	11.05a	11.91a
100	7.83ba	7.67bc	11.33a	12.07a
150	7.09b	6.85c	10.87a	11.23ba
Chilling (Positive control)	8.80a	8.80ba	11.10a	11.10ba

Means followed by the same letter are not significantly different at $P \leq 0.05$, according to Duncan's Multiple Range Test.

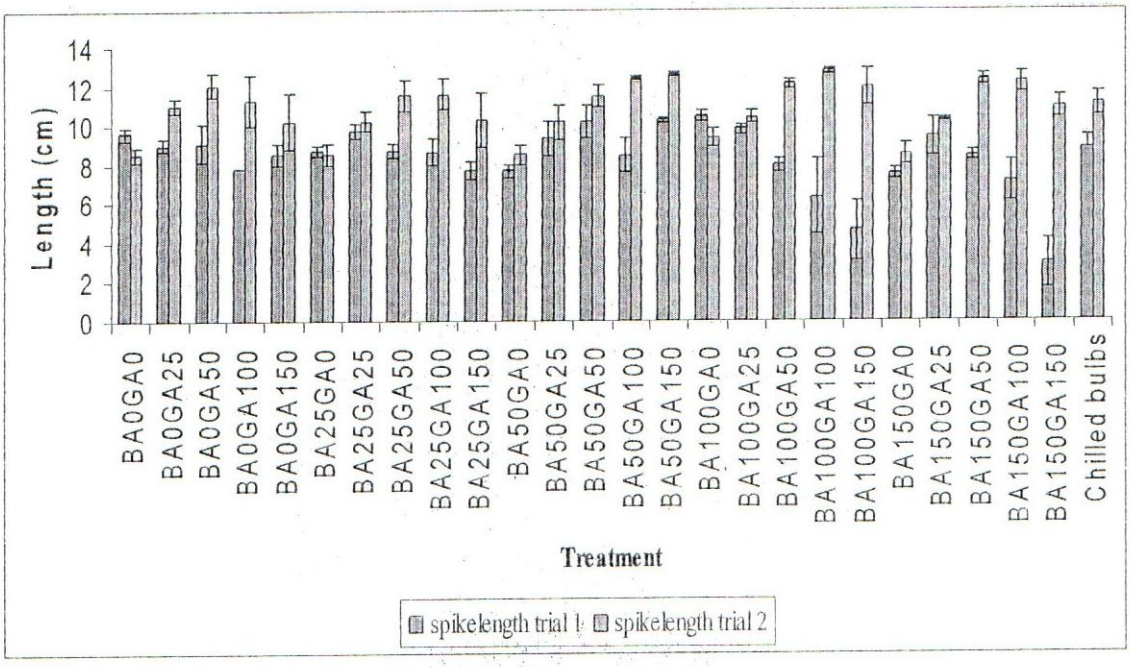

Figure 4: Influence of various combination of benzyl adenine (BA) and gibberellic acid (GA_3) on the rachis length per stem of Oriental lily bulbs *(Lilium* spp).

[(1=0,0; 2=0,25; 3=0,50 ;4=0,100 ;5=0,150; 6=25, 0; 7= 25, 25; 8=25, 50; 9=25,100; 10= 25,150; 11=50, 0; 12=50, 25; 13=50, 50; 14=50,100; 15=, 50,150; 16=100, 0; 17=100, 25; 18=100, 50; 19=100,100; 20=100,150; 21=150, 0; 22=150, 25; 23=150, 50; 24=150,100; 25=150,150; mg/l; 26= chilled bulbs]

4.4.3 Time to flowering

In general varying levels of individual PGR treatments did not significantly affect the time taken to flowering in the treated bulbs. In all single PGR treatments the number of days taken for

bulbs to flower was statistically the same and also did not differ from the controls. However, in trial 1 BA concentrations did not significantly affect the number of days to flowering

In trial 2, BA at 50 mg/l gave a mean of 127 days to flowering which was significantly different from the chilled bulbs (129.33 days). This result is in line with studies done on *Zantedeschia elliottiana* whereby Benzyl adenine accelerated flowering when the rhizome was soaked in BA solution (50-100 mgdm-3) (Tjia, 1986). Earlier flowering was also noticed in BA treated Bougainvillea 'Taipei Red' (Liang and Chang, 1998). Studies by Janowaska *et al.*, 2009 show that the use of benzyladenine accelerated the flowering of the poppy anemone.

In both trials, GA_3 had a significant influence on the number of days to flowering of Oriental lily bulbs. In trial 1 earlier flowering of 131.33 days to flowering resulted in the treatment of 50 mg/l GA_3 followed by 25 mg/l GA_3. In trial 2 (Table 7) GA_3 at 100 mg/l resulted in a mean of 125.27 days and 124.60 days was observed with GA_3 150 mg/l. A similar response of decrease in number of days to flowering was observed in hyacinth bulbs treated with GA_3 (Saniewski *et al,* 1977). The highest number of days to flowering was observed with the untreated control (133. days). ombining the plant growth regulators significantly influenced the number of days taken to flowering. BA at 25 mg/l combined with GA_3 150 mg/l significantly decreased the number of days to flowering. Similar observations were made in the combinations of benzyl adenine 50 mg/l and gibberellic acid at 100 mg/l as well as 50 mg/l BA with 150 mg/l GA_3 and 100 mg/l BA with 100 mg/l GA respectively. In most species, the transition to floral development is stimulated by GAs (Sun and Gubler, 2004). However the actual effect of GAs on flowering is still not clear because studies done by Ben-Tal and Erner (1999) reported that GAs had effects on flowering date in many plant species either becoming earlier in some plant or delayed flowering in other plants. From the study it can be concluded that plant growth regulators GA_3 and BA can be used to promote early flowering in Oriental lily bulbs and hence growers can target specific markets such as Easter and Mothers day.

Combination of the highest levels of growth regulators (150 mg/l BA and 150 mg/l GA_3) increased the number of days to flowering to 135 days in trial 1.

Table 8: Number of days to flowering of Oriental lily bulbs under different BA and GA_3 concentration treatments.

Growth regulator treatments (mg/l)	Trial 1		Trial 2	
	BA	GA_3	BA	GA_3
0 (negative control)	132.53a	132.13ba	127.60b	133.13a
25	131.73a	131.87ba	127.40b	128.27b
50	131.80a	131.33b	126.73b	126.40c
100	131.67a	132.13ba	127.73b	125.27dc
150	132.73a	133.00a	128.20ba	124.60d
Chilling(Positive control)	130.00b	130.00c	129.33a	129.33b

Means followed by the same letter are *not significantly different* at $P \leq 0.05$, according to Duncan's Multiple Range Test

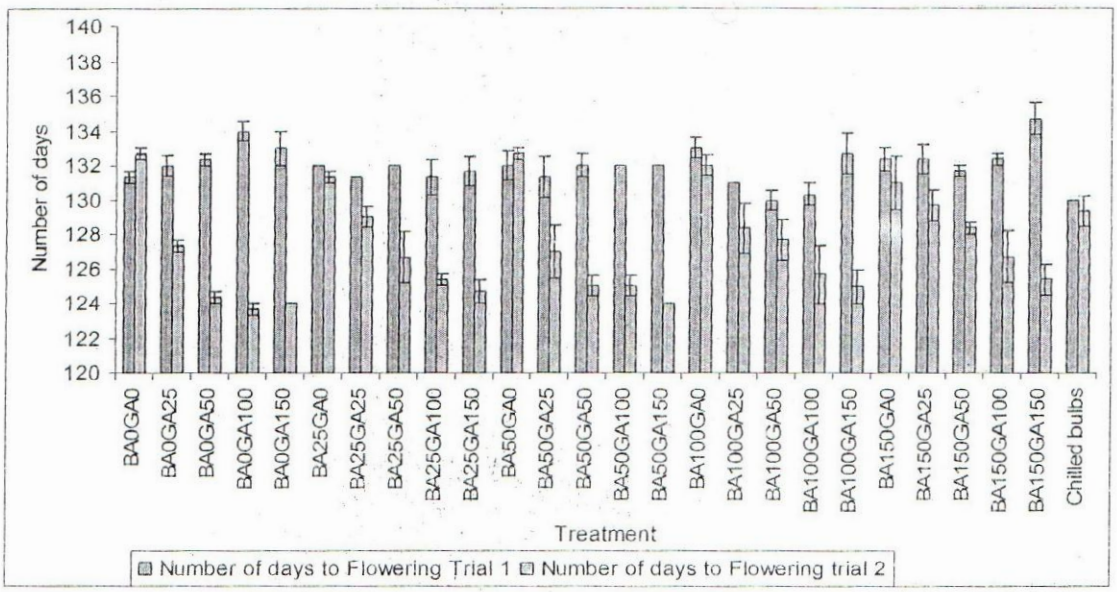

Figure 5: Influence of various combination of benzyl adenine (BA) and gibberellic acid (GA_3) on the time (number of days) taken to flowering in Oriental lily bulbs *(Lilium* spp).

[(1=0,0; 2=0,25; 3=0,50 ;4=0,100 ;5=0,150; 6=25, 0; 7= 25, 25; 8=25, 50; 9=25,100; 10= 25,150; 11=50, 0; 12=50, 25; 13=50, 50; 14=50,100; 15=, 50,150; 16=100, 0; 17=100, 25; 18=100, 50; 19=100,100; 20=100,150; 21=150, 0; 22=150, 25; 23=150, 50; 24=150,100; 25=150,150; mg/l; 26= chilled bulbs)]

4.5 Effect of benzyl adenine and gibberellic acid on the multiplication of Oriental lily bulbs

4.5.1 Bulb Weight

The bulb weight was taken 2 weeks after harvest of the lily flowers. In both trials (Table 9) BA and GA_3 treatments significantly affected the bulb weight of Oriental lily. Among the PGR treatments the highest bulb weight was recorded with BA at 100 mg/l (44.52g) which was significantly different from the other treatments.and in trial 2, maximum bulb weight was recorded with BA 50 mg/l (43.29g) which was significantly different from BA at 100 mg/l and 150 mg/l respectively. GA_3 at 50 mg/l gave the maximum bulb weight of (41.10g) and (44.84g) in both trials respectively. In trial 2, GA_3 at 50 mg/l also gave maximum weight which was statistically similar to chilled bulbs (positive control.

In trial 1 there was significant interactive influence of the two PGRs. BA at 100 mg/l combined with GA_3 at 25 mg/l resulted in a high bulb weight (52.98g). On the other hand GA_3 at 100 mg/l combined with BA 25 mg/l resulted in very low bulb weights (17.28 g). In trial 2 the interaction had a significant effect which was observed on the bulb weight. An increase in weight (50.89g) was observed when 50 mg/l BA was combined with 0 mg/l GA_3 and a decrease in weight (33.83g) when 25 mg/l GA_3 was combined with 50 mg/l BA and an increase in weight when BA 150 mg/l was combined with GA_3 50 mg/l.

GA_3 at 50 mg/l also gave maximum weight which was statistically similar to chilled bulbs (positive control). Similarly BA at 50 mg/l gave the maximum weight; Consequently, this increases the potential for larger and earlier maturing bulbs.

The size of the bulb used for planting is very important in the selection of planting materials for bulb production. This is because bulb size influences vegetative growth both in the hyacinth and the lily. In general, as the size of the planted bulb increased, vegetative growth (leaf length, width, area and plant height) also increased. A similar observation was made by Rees (1969) in tulips.

Large bulbs are expected to have larger amounts of carbohydrates and other reserves than smaller bulbs, the former should therefore have better growth and development than the latter. Flowers, florets or inflorescence are important sink organs in flowering bulb that depend on the

reserves stored in the bulb for their initial growth and development. Large bulbs have higher reserves than small bulbs and therefore plants produced from the former should have better flower quality than small bulbs.

Table 9: Bulb weight of Oriental lily bulbs under different BA and GA_3 concentration treatments.

Means followed by the same letter are not significantly different at $P \leq 0.05$, according to

	Trial 1		Trial 2	
Growth regulator treatments (mg/l)	BA	GA_3	BA	GA_3
0 (negative control)	32.76b	27.93c	42.29ab	45.49a
25	27.95c	36.02cb	41.69b	37.92c
50	29.65c	41.10b	43.29a	44.84a
100	44.52b	30.26c	37.75d	40.67b
150	32.77c	32.33cb	39.45c	35.56d
Chilling(Positive control)	54.20a	41.67a	41.67a	41.67b

Duncan's Multiple Range Test

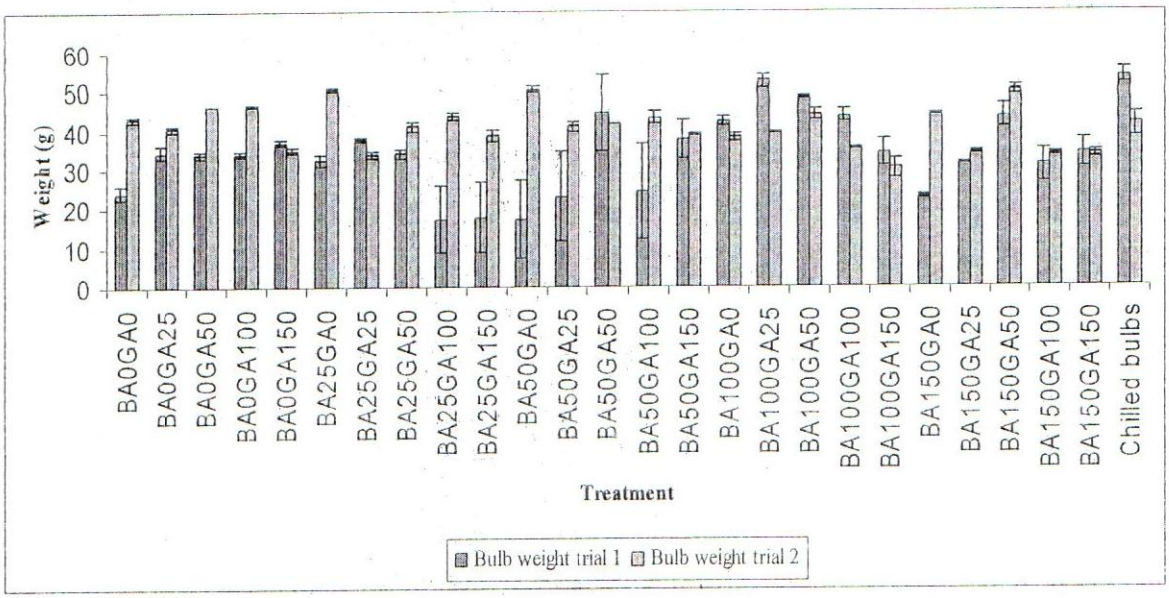

Figure 6: Influence of various combination of benzyl adenine (BA) and gibberellic acid (GA_3) on the bulb weight of Oriental lily bulbs *(Lilium* spp).

[(1=0,0; 2=0,25; 3=0,50 ;4=0,100 ;5=0,150; 6=25, 0; 7= 25, 25; 8=25, 50; 9=25,100; 10= 25,150; 11=50, 0; 12=50, 25; 13=50, 50; 14=50,100; 15=, 50,150; 16=100, 0; 17=100, 25; 18=100, 50; 19=100,100; 20=100,150; 21=150, 0; 22=150, 25; 23=150, 50; 24=150,100; 25=150,150; mg/l; 26= chilled bulbs)]

4.5.2 Number of bulblets

There was significant effect of Benzyl adenine on bulblet formation of Oriental lily bulbs in trial 1(Table 10). Benzyl adenine at 100 mg/l had one bulblet formed which was significantly different from all the other treatments which had no bulblets formed. In trial 2, both benzyl adenine and gibberellic acid had significant influence on bulblet formation. The highest number of bulblets formed was observed with BA at 100 mg/l (2.60) followed by 150 mg/l (2.33) which was significantly different from the chilled bulbs (positive control). The possible explanation for increased bulb production might be due to increased maximum carbohydrate which was translocated to the bulb for storage owing to the fact that cytokinins promote development of bulblets.

The interaction of BA and GA_3 had a significant effect on the bulblets formed; GA_3 at 150 mg/l increased the number of bulblets formed so did BA 100 mg/l combined with GA_3 25 mg/l. However at low concentration (BA 25 mg/l with GA_3 25 mg/l) resulted in very few bulblets being formed (figure 10). GA_3 at 150 mg/l gave the highest number of bulblets (2.93) followed by GA_3 50 mg/l (2.4) which was not significantly different from each other. This present results are similar to findings reported by Rana *et al.* (2005) in gladiolus and Singh (1999) in tuberose. This could be attributed to high concentration of GA_3 stimulating sink activity of developing bulbs at the expense of flower spike or inflorescence (Rana *et al* 2005).

Table 10: Influence of Benzyl adenine and gibberellic acid on the bulblets formation.

Growth regulator	Trial 1		Trial 2	
treatments (mg/l)	BA	GA_3	BA	GA_3
0 (negative control)	0.00b	0.00a	2.20ba	1.53c
25	0.13b	0.93a	1.13bc	1.33c
50	0.13b	0.53a	1.87ba	2.40ab
100	1.33a	0.40a	2.60a	1.93bc
150	0.40b	0.13a	2.33ba	2.93a
Chilling(Positive control)	0.33b	0.33a	0.33c	0.33c

Means followed by the same letter are not significantly different at $P \leq 0.05$, according to Duncan's Multiple Range Test

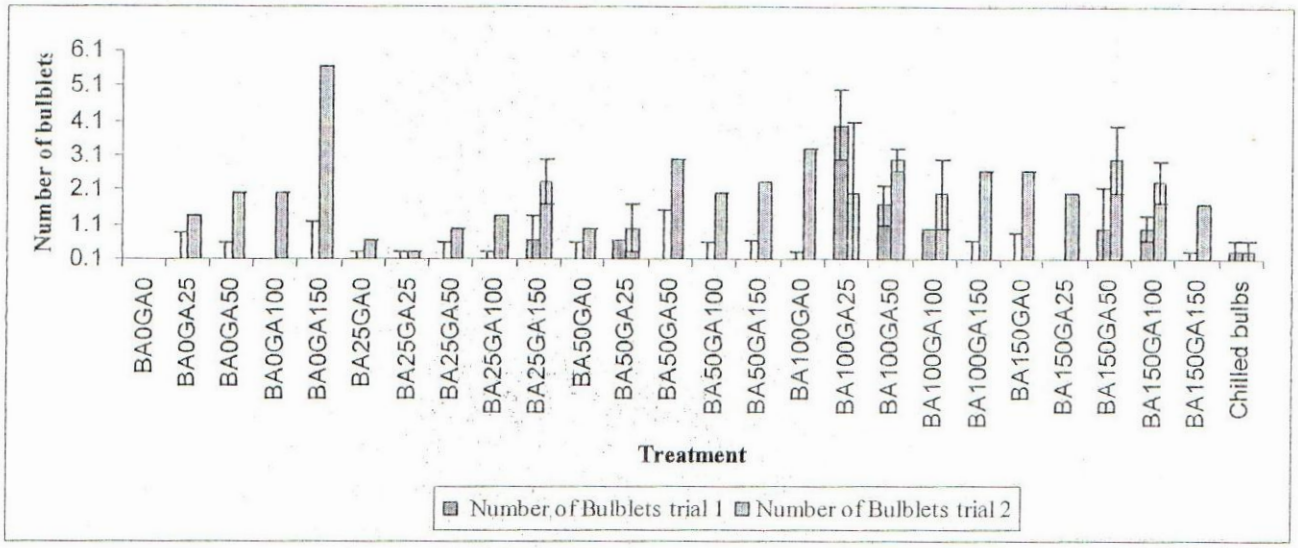

Figure 7. Influence of various combination of benzyl adenine (BA) and gibberellic acid (GA_3) on the number of bulblets of Oriental lily bulbs *(Lilium* spp) evaluated in 2009/2010.

(1=0,0; 2=0,25; 3=0,50 ;4=0,100 ;5=0,150; 6=25, 0; 7= 25, 25; 8=25, 50; 9=25,100; 10= 25,150; 11=50, 0; 12=50, 25; 13=50, 50; 14=50,100; 15=, 50,150; 16=100, 0; 17=100, 25; 18=100, 50; 19=100,100; 20=100,150; 21=150, 0; 22=150, 25; 23=150, 50; 24=150,100; 25=150,150; mg/l; 26= chilled bulbs)

4.6 Economic Analysis

When yields of Oriental lily bulbs are converted to returns, partial budget analysis reveals that in both trials PGRs were the most profitable to apply under optimistic, expected and pessimistic yield scenarios. The higher relative profitability of BA and GA_3 in trials 1 and 2 respectively was mainly due to the higher yields and low cost of production. Profitability of local cutflower production is important if producers are to remain in business. There is a growing need for highly profitable production methods in order to maximize returns to investment. In this study, returns from the use of PGRs were the most profitable in both trials. The relative profitability advantage of using PGRs is due to the generally low cost of production. In addition to the direct economic advantage of using PGRs, BA and GA_3 can be used to improve the growth and flowering of Oriental lily bulbs by increasing the percentage sprout, increasing the stem length,promoting early maturity of the flowers and intensifying the colour of florets.

The combination of economic as well as improvement of growth and flowering of Oriental lily to the small scale growers makes use of PGRs an attractive option for the production of Oriental lily in Kenya with the growers being assured of exceptionally good results.

Table 11: Production Costs (Ksh) of Oriental lily bulbs when using BA and GA_3

	Cost of production for 6.12m^2 (Ksh)		**Cost of production for 10,000m^2 (Ksh)**	
	Treatments			
Input	Plant Growth regulator	Chilling	Plant Growth regulator	Chilling
Bed Preparation (ploughing @ 7,000/ha; harrowing @ 3,000/ha)	200	200	13,000	13,000
Posts (@ 150 shs)	(4x150) = 600	600	150 shs x 1,225 posts = 183,750	183,750
Nylon net @ 130 ksh/m^2	520	520	1,300,000	1,300,000
Cost of 260 unchilled Oriental lily bulbs @ Ksh.40/bulb	10,400	10,400	42,484 bulbs x 40= 1,699,346	1,699,346
Fertilizers application (M.A.P + Manure) @ksh 4/m^2	25	25	40,000	40,000
Labour for fertilizer application	200 (1 man-day)	200	800 (4 man-days)	800
Plant growth regulator (BA&GA_3)	200	0		0
GA_3 1g for 260 bulbs@ksh 80/g	80		42,484 bulbs (160g x 80) 12800	0
3g BA for 260 bulbs @ Ksh 140/g	140		42,484 bulbs (160g x 140) 22400	0
Labour for PGR.application	200 (1 man-day)		800 (4 man-days)	0
Electricity costs for chilling 260 (6.12m^2) to 424,840 (10,000m^2) bulbs over 8 weeks	0	10000	0	80,000
Cold store labour	200 (1 man-day)	200 (1 man-day)	800 (4 man-days)	4 man days (200 (x4 tons/ha))= 3200

Planting labour	200 (1 man-day)	200 (1 man-day)	800 (4 man-days)	800
Cost of Pesticides	130	130		212,418
Spraying Labour	200 (1 man-day)	200 (1 man-day)	800 4 man-days)	800
Weeding Labour	200 (1 man-day)	200 (1 man-day)	4 man-days 800	800
Post harvest	1200	1200	1200	1200
Post harvest labour	200	200	800	800
Total Production Costs	**15,135**	**24,075**	**3,490,514**	**3,536,914**

Table 12 a :Returns (Ksh)in Trial 1

Cost of production for 6.12m^2 (Ksh)		**Cost of production for 10,000m^2 (Ksh)**	**Cost of production for 6.12m^2**	**Cost of production for 10,000m^2 (Ksh)**
Trial 1	Hormone Treated bulbs	Chilled bulbs	Hormone Treated bulbs	Chilled bulbs
	Optimistic Yields (Experimental plots)		**Optimistic Yields (Experimental plots)**	
Yield	570.00	570.00	931,373.00	1,209,150.00
Gross returns	34,200.00	34,200.00	55,882,380.00	96,732,000.00
Production costs	15,135.00	24,075.00	3,490,514	3,490,514
Returns	**19,065.00**	**10,125.00**	**52,391,866.00**	**93,241,486.00**
	Expected Yields (80% of Experimental plots)		**Expected Yields (80% of Experimental plots)**	
Yield	456.00	456.00	745,098.40	745,098.40
Gross returns	27,360.00	27,360.00	44,705,904.00	44,705,904.00
Production costs	12,108.00	19,260.00	2,792,411.20	2,829,531.20
Returns	**15,252.00**	**8,100.00**	**41,913,492.80**	**41,876,372.80**
	Pessimistic yields (60% of Experimental plots)		**Pessimistic yields (60% of Experimental plots)**	
Yield	342.00	342.00	558,823.80	558,823.80
Gross returns	20,520.00	20,520.00	33,529,428.00	33,529,428.00
Production costs	9,081.00	14,445.00	2,094,308.40	2,122,148.40
Returns	11,439.00	6,075.00	31,435,119.60	31,407,279.60

Table 12 b :Returns(Ksh) in Trial 2

Returns(Ksh) in Trial 2	Cost of production for 6.12m^2 (Ksh)	Cost of production for 10,000m^2 (Ksh	Cost of production for 6.12m^2 (Ksh)	Cost of production for 10,000m^2 (Ksh
	Hormone Treated bulbs	Chilled bulbs	Hormone Treated bulbs	Chilled bulbs
	Optimistic Yields(Experimental plots)		**Optimistic Yields(Experimental plots)**	
Yield	740.00	740.00	1,209,150.00	931,373.00
Gross returns	59,200.00	59,200.00	96,732,000.00	55,882,380.00
Production costs	15,135.00	24,075.00	**3,490,514**	**3,536,914**
Returns	44,065.00	35,125.00	93,241,486.00	52,345,466.00
	Pessimistic Yields(80% of Experimental plots)		**Pessimistic Yields(80% of Experimental plots)**	
Yield	592.00	592.00	967,320.00	745,098.40
Gross returns	47,360.00	47,360.00	77,385,600.00	44,705,904.00
Production costs	12,108.00	19,260.00	2,792,411.20	2,829,531.20
Returns	35,252.00	28,100.00	74,593,188.80	41,876,372.80

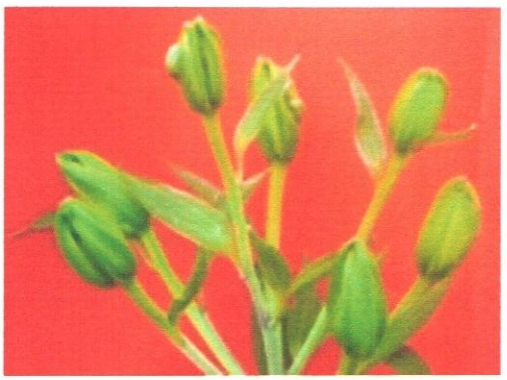

Plate 3: Small buds due to high concentrations Levels of BA and GA_3

Plate 4: Floret at actual harvest stage

Plate 5: Good quality buds with GA_3 50mg/l

Plate 6: Sprout emergence on lily bulb

Plate 8: Bulblets formed from mother bulb

Plate 7: Early flowering due to high levels of PGRs

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The study involved evaluating the effects of BA and GA_3 on the breaking of dormancy in Oriental lily bulbs pre-soaked in different concentration levels of the two PGRs before planting. In this present study it is clear that benzyl adenine and gibberellic acid can be used as an alternative treatment to low temperature in breaking of dormancy in Oriental lily bulbs. Both BA and GA_3 at 50 mg/l concentration resulted in highest percentage of sprouting, a reduction in time to 50% sprout emergence, high number of leaves, earlier flowering, and longer flower stems. BA at 100 mg/l and GA_3 at 150 mg/l gave the highest number of bulblets. Combined effects of BA and GA_3 reduced number of days to opening of the first leaf whorl, decreased the number of days to flowering, promoted a high number of florets, and increased the rachis length Therefore, lily growers can maximize their profit margins by using PGRs to substitute low temperature treatment procedure for breaking dormancy.

The use of plant growth regulators is a cost-effective method in breaking dormancy in Oriental lily bulbs this was confirmed in the present study after a cost benefit analysis was done.

5.2 Recommendations

Based on the findings of the present study, the following recommendations can be made:

1. BA and GA_3 should be used in Oriental lily production. Low concentrations of 50 mg/l of BA and GA_3 singly and BA 100 mg/l plus GA_3 50 mg/l in combination proved to be the best for the growth and flowering of Oriental lily bulbs.
2. Further studies need to be carried out to determine the time taken between lifting of the bulbs and dormancy breaking treatment.
3. Further studies is required to investigate the effects of pre and post-PGR treatments in combination with chilling at different durations on the performance of Oriental lily.

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APPENDICES

Appendix 1. ANOVA Table for effects of BA and GA_3 on percent sprout emergence

Trial 1

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	287.18	143.59	0.51	0.6015
BA	4	9005.33	2251.33	8.05	<.0001
GA	4	5762.00	1448.00	5.18	0.0014
BA*GA	16	7914.67	494.67	1.77	0.0635
Error	50				

Trial 2

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	1286.54	643.27	5.61	0.0063
BA	4	13741.33	3435.33	29.68	<.0001
GA	4	7221.33	1805.33	15.75	<.0001
BA*GA	16	1158.67	72.42	0.63	0.8425
Error	50				

Appendix 2. ANOVA table for effects of BA and GA_3 on number of days to 50% sprout emergence

Trial 1

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	13.64	6.82	0.28	0.7583
BA	4	584.85	146.21	5.96	0.0005
GA	4	637.52	159.38	6.5	0.0003
BA*GA	16	2007.55	125.47	5.12	<.0001
Error	50				

Trial 2

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	2.87	1.44	1.16	0.3212
BA	4	86.35	21.59	17.47	<.0001
GA	4	7.55	1.89	1.53	0.2087
BA*GA	16	19.25	1.2	0.97	0.498
Error	50				

Appendix 3: ANOVA table for effects of BA and GA_3 on number of days to opening of the first leaf whorl

Trial1

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	46.33	23.17	1.16	0.3224
BA	4	68.32	17.08	0.85	0.4982
GA	4	183.25	45.81	2.29	0.0726
BA*GA	16	1000.75	62.55	3.13	0.001
Error	50	46.33	23.17	1.16	0.3224

Trial2

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	11.49	5.74	2.41	0.1002
BA	4	57.6	14.4	6.04	0.0005
GA	4	221.33	55.33	23.21	<.0001
BA*GA	16	171.07	10.69	4.49	<.0001
Error	50				

Appendix 4: ANOVA table for effects of BA and GA_3 on number of leaves

Trial 1

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	7.43	3.72	0.3	0.7442
BA	4	211.03	52.76	4.22	0.0051
GA	4	127.04	31.76	2.54	0.051
BA*GA	16	535.17	33.45	2.68	0.0041
Error	50				

Trial2

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	163.15	81.57	8.23	0.0008
BA	4	304.83	76.21	7.69	<.0001
GA	4	73.54	18.38	1.86	0.133
BA*GA	16	976.86	61.05	6.16	<.0001
Error	50				

Appendix 5: ANOVA table for effects of BA and GA_3 on the stem length

Trial 1

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	688.71	344.36	10.13	0.0002
BA	4	1289.51	322.38	9.48	<.0001
GA	4	924.32	231.08	6.8	0.0002
BA*GA	16	3137.27	196.08	5.77	<0.0001
Error	50				

Trial 2

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	65.89	32.94	2.84	0.0679
BA	4	192.93	48.23	4.16	0.0055
GA	4	168.72	42.18	3.64	0.0112
BA*GA	16	542.23	33.89	2.92	0.0019
Error	50				

Appendix 6: ANOVA table for effects of BA and GA_3 on the number of florets

Trial 1

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	5.77	2.88	1.47	0.2401
BA	4	176.88	44.22	22.51	<0.0001
GA	4	123.41	30.85	15.7	<0.0001
BA*GA	16	157.79	9.86	5.02	<0.0001
Error	50				

Trial 2

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	1.75	0.88	3.43	0.0401
BA	4	4.75	1.19	4.65	0.0029
GA	4	4.76	119	4.66	0.0028
BA*GA	16	7.67	0.48	1.88	0.0465
Error	50	1.75	0.88	3.43	0.0401

Appendix 7: ANOVA table for effects of BA and GA_3 on the rachis length

Trial 1

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	9.15	4.58	2.48	0.0938
BA	4	43.83	10.96	5.94	0.0005
GA	4	66.2	16.55	8.97	<.0001
BA*GA	16	104.35	6.52	3.54	0.0003
Error	50				

Trial 2

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	22.97	11.49	11.59	<.0001
BA	4	6.96	1.74	1.76	0.1528
GA	4	113.64	28.41	28.67	<.0001
BA*GA	16	15.18	0.85	0.96	0.5144
Error	50		11.49	11.59	<.0001

Appendix 8: ANOVA table for effects of BA and GA_3 on the number of days to flowering

Trial 1

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	4.41	2.21	1.79	0.1775
BA	4	15.01	3.75	3.05	0.0253
GA	4	21.81	5.45	4.43	0.0038
BA*GA	16	45.52	2.85	2.31	0.0125
Error	50				

Trial 2

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	17.33	8.67	3.67	0.0325
BA	4	17.2	4.3	1.82	0.1393
GA	4	703.87	175.97	74.56	<.0001
BA*GA	16	170.93	10.68	4.53	<.00001
Error	50				

Appendix 9: ANOVA table for effects of BA and GA_3 on the bulb weight

Trial 1

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	257.05	128.53	1 58	0.2154
BA	4	2522.2	630.55	7.77	<.0001
GA	4	1603.96	400.99	4.94	0.002
BA*GA	16	2585	161.56	1.99	0.0329
Error	50				

Trial 2

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	0.94	0.97	0.24	0.7887
BA	4	296.21	74.05	18.26	<.00001
GA	4	1081.4	270.35	66.65	<.00001
BA*GA	16	899.1	56.19	13.85	<.00001
Error	50				

Appendix 10: ANOVA table for effects of BA and GA_3 on the bulblet

Trial 1

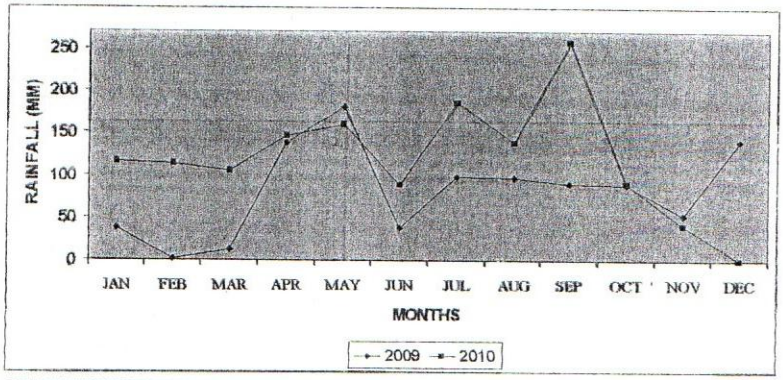
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	5.02	2.51	3.02	0.06
BA	4	17.6	4.4	5.28	0.0013
GA	4	8	2	2.4	0.062
BA*GA	16	30.4	1.9	2.28	0.014
Error	50				

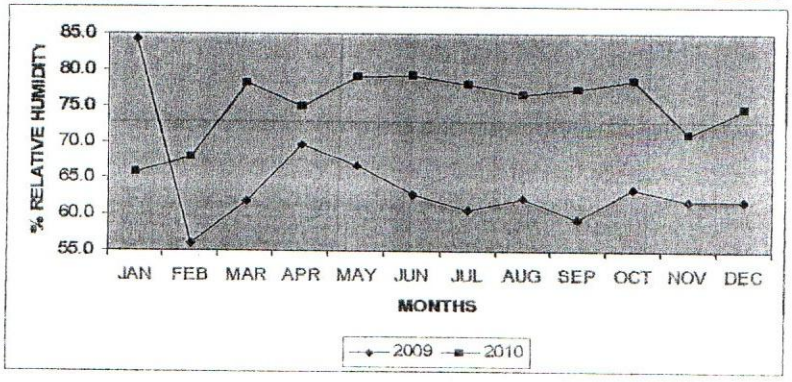
cv=

Trial 2

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F Value	Probability
Replication	2	0.31	0.15	0.11	0.8938
BA	4	19.15	4.79	3.5	0.0135
GA	4	25.41	6.35	4.65	0.0029
BA*GA	16	51.39	3.21	2.35	0.011
Error	50				

4.8 Weather Data





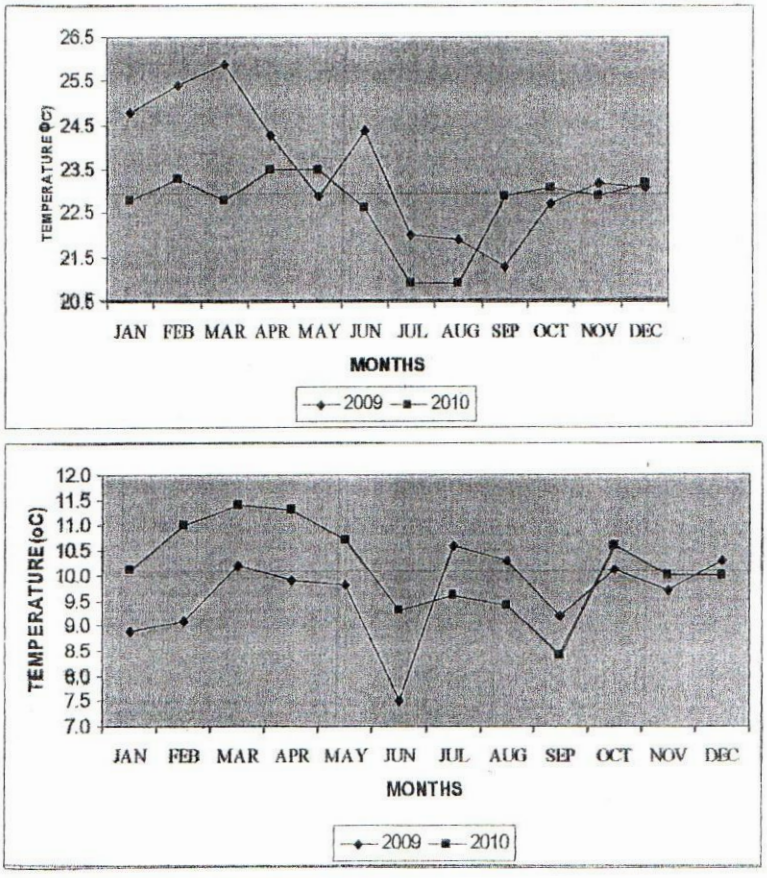


Figure 8: Rainfall pattern in the year 2009-2010 at Lemotit farm

Trial 1: September 2009-January 2010

Trial 2: January 2010-April 2010

YEAR	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Rainfall												
2009	39.0	2.5	13.0	138.3	180.7	38.5	98.5	97.1	90.5	89.0	52.6	140.5
2010	116.1	114.5	105.7	146.8	159.3	88.7	186.0	137.4	256.8	89.4	40.8	0.00
2009 Temp												
Max	24.8	25.4	25.9	24.3	22.9	24.4	22.0	21.9	21.3	22.7	23.2	23.1
Min	8.9	9.1	10.2	9.9	9.8	7.5	10.6	10.3	9.2	10.1	9.7	10.3
2010												
Max	22.8	23.3	22.8	23.5	23.5	22.6	20.9	20.9	22.9	23.1	22.9	23.2
Min	10.1	11.0	11.4	11.3	10.7	9.3	9.6	9.4	8.4	10.6	10.0	10.0
%RH												
2009	84.3	56.1	61.8	69.6	66.7	62.8	60.6	62.1	59.4	63.5	61.8	61.8
2010	65.8	68	78.2	74.9	79.1	79.2	78.0	76.6	77.3	78.6	70.9	74.6

Trial 1: September 2009-January 2010
Trial 2: January 2010-April 2010