EFFECT OF BENZYLADENINE AND GIBBERELLINS ON YIELD COMPONENTS, YIELD AND THE POSTHARVEST SHELF LIFE OF FRENCH BEANS (*Phaseolus vulgaris* L.)

BY

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A56/7550/97

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE DEGREE OF

MASTER OF SCIENCE

IN

HORTICULTURE

FACULTY OF AGRICULTURE,
DEPARTMENT OF CROP SCIENCE,
UNIVERSITY OF NAIROBI.

2001

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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DEDICATION

This thesis is dedicated to:

The entire family of Mr. and Mrs. Jonathan Arthur Ambuko.

Special dedication goes to my 'twin' sister Winfred Opisa, who was used of God to rescue me from the cruel hand of death by donating one of her kidneys to me.

ACKNOWLEDGEMENTS

My sincere gratitude goes to my supervisors Dr. Vallantino Emongor and Dr. Solomon I. Shibairo for their assistance and guidance in the course of the study and thesis write up. I am heavily indebted to Dr. Emongor, who freely provided the Plant Growth Regulators used in this study. May the lord God richly bless him.

I would like to sincerely thank the University of Nairobi for awarding me a scholarship to pursue a postgraduate course. My appreciation goes to the Chairman and the technicians in Crop Science Department for allowing me to use the facilities in the department to carry out my research.

I greatly appreciate my family members, who continually encouraged me to press on. I also appreciate my colleagues and friends like Juma, Mugarura, Muchiri, Maina, Catherine, Shem and many others not mentioned by names for their continued encouragement and support. I highly appreciate the Mugabe hall postgraduate fellowship for their continued prayers. Last but not least, I thank the almighty God for his enabling grace, without him I would not have achieved anything.

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LIST OF ABBREVIATIONS

a.i:	Active ingredient
ABA:	Abscisic Acid
ANOVA:	Analysis of Variance
BA:	Benzyladenine
BAP:	Benzylaminopurine
chl.:	Chlorophyll
cm:	Centimeters
CO_2:	Carbon dioxide
Conc.:	Concentration
DAP:	Diammonium phosphate
GA_3:	Gibberellic acid
GA_{4+7}	Gibberellins 4 and 7
ha:	Hectare
HCDA:	Horticultural Crops Development Authority
IAA:	Indole-3-acetic acid
K:	Potassium
l:	Litre
LSD:	Least Significant Difference
M:	Molar
mg:	Milligrammes
ml:	millilitre

mM:	Millimolar
N:	Nitrogen
NAA:	1- Naphthalene acetic acid
No.:	Number
NPK:	Nitrogen, Phosphorus, Potassium.
^{o}C:	Degrees celsius
P:	Phosphorus
PGRs:	Plant growth regulators
tons:	Tonnes

ABSTRACT

The objective of this study was to investigate the effect of exogenous gibberellins (GA_{4+7}) and Accel on yield components, yield and postharvest shelf life of French beans. The plants were sprayed to run-off with four concentrations of GA_{4+7} (1.25, 2.50, 3.75 or 5.0 mg/litre), Accel (12.5, 25.0, 37.5 or 50.0 mg/litre) and a control (plain water) just before flowering.

Both GA_{4+7} and Accel significantly increased the pod number, fresh pod yield, pod length and water content of the pods. Independently BA did not have any significant effect on pod length, pod number per plant or fresh pod yield, suggesting that the effect of Accel on pod length, pod number, fresh pod yield and water content of the pods was a GA_{4+7} effect rather than a BA effect. Accel in experiment 1 and GA_{4+7} in experiment 2 reduced wilting of the pods. Both Accel and GA_{4+7} reduced the rate of post storage water loss by the pods; thus slowing down wilting which is characteristic of fresh produce such as French beans.

Accel increased the chlorophyll content of French bean pods and leaves and enhanced chlorophyll retention by the pods after storage thereby slowing down yellowing or senescence of the pods. Gibberellins (GA_{4+7}) did not have a significant effect on the chlorophyll content of the leaves and the pods, hence the increase of both leaf and pod chlorophyll content was attributed to BA. Neither GA_{4+7} nor accel affected the rate of water loss by the pods after cold storage and none of the two plant growth regulators imparted chilling-hardiness to French bean pods. The author concludes that Accel and

GA_{4+7} at lower concentrations increased the yield, quality and postharvest shelf life of French bean pods. This suggests that Accel and GA_{4+7} have the potential to be used to improve the agronomy of French beans in Kenya.

CHAPTER ONE

INTRODUCTION

1.1 The Horticultural Industry in Kenya

Over the past two decades, the horticultural industry in Kenya has expanded tremendously becoming a major contributor to the country's foreign exchange earning and a leading employer due to its labour intensiveness (Kibanga, 1996). Large numbers of vegetables, cut-flowers and fruits are exported by air to Europe. The main crops are capsicums and chillies, snap beans (French beans), eggplants, courgettes (squash), okra, roses, carnations and a wide range of vegetables grown specifically for Asians. Tropical flowers, asparagus, plumosus and rooted chrysanthenum cuttings are also exported (Waithaka, 1974).

Cutflower production is the major contributor to foreign exchange in the horticultural industry in Kenya (HCDA, 1996). However, the contribution from vegetable production has continued to rise over the years with Asian vegetables, pepper and French beans, among others being of importance. French beans contribute about 50% to the total volume of vegetables exported from Kenya (HCDA, 1999).

1.2 French Beans *(Phaseolus vulgaris L.)*

The bean is of New World origin, principally South American (Kaplan, 1981). Phaseolus vulgaris L. is the best known and most widely cultivated species of Phaseolus in the world (Tripath and Singh, 1968). The common bean is the leading legume both in production and consumption (Martis, 1989). The terms French beans, snap beans,

kidney beans are all used synonymously for beans that produce pods for vegetables (Njeru, 1989). In the export market, terms such as snap beans, haricot verts, fillet beans or string beans refer to French beans (HCDA, 1996). French beans are mainly grown for immature pods which are either consumed fresh or processed and canned. French beans differ from field beans in that they possess thicker pods which are relatively free of bast fibres in the early stages of development. If this property of low fibre is to be realised, picking of pods has to be regular because fibre content increases as the pods mature. There are three grades of French beans namely extra fine, fine and bobby in decreasing order of superiority (HCDA, 1996). These grades depend not only on timely and regular picking, but also on sound crop husbandry practices such as fertilization, disease and pest control and irrigation to alleviate water stress.

1.3 French Bean Production in Kenya

In Kenya there are about ten thousand licensed vegetable and fruit exporters of which only twenty to thirty handle majority of the volume (Grisley, 1989). The export firms contract local small-scale farmers to grow French beans. The contracting firms supply the farmers with seed and technical assistance and fix produce prices beforehand. Seeds supplied by the exporting firms are typical of the varieties preferred by consumers in the importing countries. For example, French beans for French, Dutch and British markets are mostly of 'Monel' variety (Grisley, 1989).

French beans production in Kenya is mainly for export market with the major outlets being France and the United Kingdom, (HCDA, 1996). Although French bean consumption locally has been negligible, there is a steady increase in consumption,

hence a potentially good market (HCDA, 1996). French beans cultivars grown in Kenya include 'Monel', 'Espada', 'Maasai', 'Morgan' and 'Gloria' (HCDA, 1996).

1.4 Problems of French Bean Production in Kenya

There are a number of constraints facing horticultural crop production in Kenya. These problems range from low production, postharvest losses due to lack of organized market facilities.

Conventional measures to improve crop production include intensive breeding and improved crop husbandry through irrigation, fertilization, pest and disease control, use of modern machinery and equipment (Mutunga, 1998). Most technological packages have been adopted and the application in most cases has reached the economic optimum leading to diminishing returns for extra inputs. New measures are therefore necessary to generate technologies in the 21st century to meet the increasing demand for food. With the current population explosion, increased efficiency in the use of available land and other resources is of great importance.

To meet the rising demand for food, emphasis has to be put on improving yield per hectare. This is because the scope to increase cropped area is limited and often results in environmental degradation (Mutunga, 1998). It is therefore necessary to try new, unexplored technological packages to improve crop production and reduce postharvest losses. One such package is the use of plant growth regulators (PGRs) to modify plant growth and development

Increased postharvest losses in French beans is a result of lack of organized market facilities such as transportation and temporary storage, lack of marketing cooperatives

and the seasonality of the time (Obara, 1991). Like other horticultural commodities, French beans are highly perishable (Ryall and Lipton, 1979). They rapidly deteriorate once they are picked unless proper handling and storage measures are undertaken (Njeru, 1989). During peak production periods (November to May), there is limited cargo space in aeroplanes. Most exporters are thus forced to temporarily hold their produce under refrigeration for a few days, while awaiting airlifting. The option to this is that they only harvest and airlift small quantities each day. Both options are expensive for the exporter. Cold storage is a very expensive venture leading to increased production costs. Cold storage may also result in chilling injury, thereby reducing the value and quality of the produce. On the other hand, airlifting small amounts is wasteful, because once the crop starts podding, any delays in harvesting results in increased fibre content of the pods, hence a reduction in quality. As earlier noted most exporters contract farmers to produce and the produce price is fixed beforehand; therefore losses incurred due to deferred picking are solely the exporter's problem (Anon., 1998).

Given the perishability problems cited above, measures to improve or increase the shelf-life of the produce would be welcome to the those involved in French bean production. Some of the measures necessary are those that will reduce water loss by pods thereby slowing down the wilting process, improve chlorophyll retention thereby slowing down the yellowing of pods and reduce incidents of chilling injury upon cold storage.

Measures aimed at improving the shelf-life of the produce will contribute to efforts aimed at increasing crop production by reducing postharvest losses.

Cytokinins and Gibberellins (GAs) are among the phytohormones implicated in almost

all stages of plant growth and development. These phytohormones have been shown to affect plant growth right from seed germination to floral induction to senescence.

Some of the known effects of GAs include cell elongation, leading to elongated plant tissues; promoting fresh weight; overcoming dormancy in seeds and buds; inducing flowering and parthenocarpic fruit development in some species e.g. grapes and pears (Emongor 1995; Hallman, 1990; Salisbury and Ross, 1990). GAs are also known to stimulate mobilization of foods and minerals in storage cells, especially cereal grains (Akazawa and Miyata, 1982).

Cytokinins are known to enhance cell division (cytokinesis) and enlargement (Salisbury and Ross, 1990). Cytokinins promote carbohydrate metabolism and create new source-sink relationship, thereby leading to increased dry matter accumulation in the sinks (Dyer *et al.*, 1990). Cytokinins have been shown to delay senescence (Mooney and Van Staden, 1986) by enhancing retention of chlorophyll and delaying yellowing of detached tissues such as leaves (Dyer and Osborne, 1971; Sabaster, 1985). Delay of senescence by cytokinins has been explored and largely exploited in the cut-flower industry. Cytokinins are used as anti-senescence additives in the formulation of cut-flower holding solutions. Cytokinins' influence on cell division has been exploited in the *in vitro* mass propagation. The role of PGRs on growth, yield and the shelf-life of most food crops is yet to be sufficiently explored.

OBJECTIVES

The main objective of this study was to enhance yield components, yield and to prolong the post harvest shelf-life of French beans, using gibberellins and cytokinins.

The specific objectives were:

1. To determine the effect of benzyladenine N-(phenylmethyl)-1H-purine-6-amine) on yield, yield components and postharvest shelf-life of French beans

2. To determine the effect of gibberellins (GA_{4+7}) on yield, yield components and postharvest shelf-life of French beans

CHAPTER TWO

LITERATURE REVIEW

2.1 Botany

Beans (*Phaseolus vulgaris* L.) belong to the family Leguminocea consisting of approximately 600 genera, within which are various species. Only 40 of the many species of beans are of economic importance to human consumption (Hawthorn, 1981). Many hundreds of bean cultivars are cultivated for their immature pods and dry or green seeds. There is no clear distinction between cultivars for pods and those for seeds, because the same cultivar may be used for both pods and seeds (Grubben, 1977).

French beans are mainly grown for the immature pods, which are either consumed fresh or processed and canned. There are two major types of French beans: 1) The dwarf or bush cultivars, which are day neutral, early maturing, 20 to 60cm in height, with lateral terminal inflorescence and determinate growth. These cultivars do not require any support (staking). 2) Climbing or pole cultivars, which have an indeterminate growth and grow up to 3 m in height; they require staking. There are both day neutral and short day cultivars within this group (Njeru, 1989).

French beans differ from field beans in that they possess thicker pods that are relatively free of bast fibres in the early stages of development. The pods are narrow and mostly glabrous, straight or curved with the colour ranging from yellow to dark green. The seeds also vary in colour from white to black (Tindall, 1983).

2.2 Ecological Requirements

In Kenya French beans can be grown in areas with an average annual rainfall ranging from 900 to 2000 mm, which should be well distributed during the growing season. Under such conditions, supplementary irrigation may be beneficial. Under drier conditions irrigation is absolutely necessary. Heavy rainfall adversely affects flower fertilization, resulting in reduced pod set (Njeru, 1989).

The ideal altitude ranges between 1500 to 2100m above sea level (HCDA, 1996). At higher altitude the growth period is prolonged and there are increased incidences of diseases, because of the colder conditions. Lower altitudes tend to have low rainfall hence are not ideal. The optimum temperature range is 16 to 24°C; below 10°C bean plants are destroyed by chilling, while at temperatures above 30°C blossom drop is very serious and may hamper pod and/or seed set (Anon., 1989).

French beans thrive in a wide range of soil types, ranging from light sand to heavy clays. The best soils for growth should be friable, well drained, loam soils with high organic matter (Tindall, 1983). The optimum soil pH is between 6.5 and 7.5, but slight acidity can be tolerated (Anon, 1989).

2.3 French Bean Production in Kenya

Although French bean production is a relatively new venture in Kenya, it has grown to be a major contributor to the fresh produce export market. The main export season for the French beans is October to May, which coincides with the winter period in Europe (HCDA, 1996). Most growers therefore, schedule their production such that the bulk of the produce is ready during the months of October to mid-December and from mid-

January to the end of May (HCDA, 1996). Both small-scale and large-scale growers undertake French bean production. Small-scale production is more prevalent and in most cases the farmers are contracted by export agents (middlemen), who provide inputs such as pesticides, fungicides and fertilisers, besides giving technical advice (Grisley, 1981). Production of French beans in Kenya occurs along riverbeds and in irrigated areas including Embu, Meru and Mwea (Eastern Province); Naivasha, Trans Nzoia and Nakuru (Rift valley Province); Bungoma and Vihiga (Western Province).

2.4 Plant Growth Regulators (PGRs)

A plant hormone (phytohormone) is an organic compound synthesised in one part of the plant and translocated to another part where at a very low concentration (<1mM) it causes a physiological response (Salisbury and Ross, 1990). The response in the target organ could be promotive, inhibitory or qualitative modification of growth and development (Moore, 1979). The commonly recognised classes of plant hormones include auxins, gibberellins, cytokinins, absissic acid, ethylene and polyamines (Salisbury and Ross, 1990).

2.5 Cytokinins

Cytokinins are substituted adenine compounds, which promote cytokinesis (cell division) in tissue grown *in vitro* (Salisbury and Ross. 1990). The first cytokinin to be isolated was kinetin Miller *et al.*, 1956). Kinetin has not so far been found in plants. Zeatin was the first naturally occurring cytokinin in plants to be isolated from corn kernels (Salisbury and Ross, 1990). Three most commonly detected and most physiologically active cytokinins include zeatin, dihydrozeatin and isopentyladenine

(IPA). Kinetin and benyladenine (BA) are highly active. Benzyladenine riboside has been extracted from petunia (Salisbury and Ross, 1990).

Cytokinins are synthesised in developing seeds, leaves and root tips (Miller, 1988). They are found in high concentrations in young organs (seeds, fruits and leaves) and in root tips. Cytokinins synthesised in the roots are translocated to other plant parts through the xylem (Salisbury and Ross, 1990). Cytokinins have been implicated in almost all stages of plant growth and development, ranging from seed germination to floral induction to senescence.

2.5.1 Physiological Roles of Cytokinins

One predominant effect of cytokinins is that of stimulating cell division and enlargement. This effect of cytokinins has been demonstrated in many plant species. When the pith from tobacco, soybean and other dicot stems is cut off and cultured aseptically on agar medium with an auxin and proper nutrients, a mass of callus is formed; if a cytokinin is provided, cytokinesis is greatly promoted. If the cytokinin: auxin ratio is maintained high, certain cells are produced in the callus which divide and give rise to others that develop into buds, stems and leaves. At low cytokinin: auxin ratio, root formation is favoured (Salisbury and Ross, 1990).

Cytokinins have been shown to promote cell elongation in young leaves, cotyledons, hypocotyls and coleoptiles of some plant species (Salisbury and Ross, 1990). Cytokinins increase cell expansion in dicot cotyledons and leaves. Incubation of cotyledons with a cytokinin was shown to enhance growth two-fold relative to controls without the hormone (Salisbury and Ross, 1990). This growth promotion has been

demonstrated in many plant species including radish, sugarbeet, sunflower, cucumber, muskmelon and lettuce. The overall growth promotion by cytokinins is a result of faster cell expansion and production of larger cells. Definite promotive effects on intact dicot leaves of some species occur after repeated applications; the effects are however small and may arise indirectly through attraction of metabolites from other organs (Salisbury and Ross, 1990).

Cytokinins have been shown to promote cell expansion in dicot cotyledons and leaves. This was seen when some or all of the roots (sites of cytokinin synthesis) were removed from the bean plant (*Phaesolus vulgaris*) and winter rye (*Secale cereale*). Leaf growth from the rootless plants was soon slowed in both species, but application of a cytokinin to the leaves restored much of the growth (Salisbury and Ross, 1990). Exogenous cytokinins can promote cell elongation in young leaves, cotyledons, coleoptiles of wheat and watermelon, but much remains to be learnt about the normal role of cytokinins in cell expansion. Exogenous cytokinin application was shown to restore growth in soybean after removal of roots, the endogenous source of cytokinins (Salisbury and Ross, 1990).

The mechanism of action by cytokinins varies in different tissues hence the variability of cytokinin effects. Fosket (1977) reported that cytokinins promote cell division by increasing the transition of cells from G_2 to mitosis, (G_2 refers to the period of cell growth after DNA replication that preceeds mitosis). Cytokinins increase the transition of cells from G_2 to mitosis by increasing the rate of protein synthesis. Some of these proteins could be enzymes needed for mitosis. Stimulating formatiom of mRNAs that code for protein could also increase protein synthesis.

Cytokinins effect seems to be specifically on translation. The ribosomes in treated cells are frequently grouped in large protein synthesizing polysomes rather than in small polysomes or as free monoribosomes characteristic of slowly dividing cells. Experimental evidence has also shown that cytokinins promote faster incorporation of radioactive amino acids into proteins and inhibition of the physiological response by inhibitors of protein synthesis.

Cytokinins effect may also be as a result of their effect on the cell's osmotic potential. When radish cotyledons are grown in weak light, cytokinins increase the internal production of reducing sugars (mainly glucose, sucrose and fructose) and these sugars act osmotically to cause water uptake that drives growth (Huff and Ross, 1975; Bewli and Witham, 1976). For radish and cucumber cotyledons, cytokinin treatment causes increased plasticity (not elasticity) of the cell walls, thus the walls become loosened so they will expand faster irreversibly under the existing turgor pressure (Thomas *et al.*, 1981). This wall loosening is deemed more important for growth promotion than enhanced turgor pressure resulting from reducing sugar production. This is because cytokinin treated cotyledons grow with only about 0.15 MPa turgor pressure compared to about 0.90 MPa for untreated cotyledons (Rayle *et al.*, 1982). The kind of wall loosening caused by cytokinins is not a result of acidification of the wall (Ross and Rayle, 1982).

2.6 Gibberellins (GAs)

Gibberellins are isoprenoid compounds. They are specifically diterpenes synthesised from acetate units of acetyl co-enzyme A by the mevalonic acid pathway (Salisbury and Ross, 1990). More than 60 GAs have been discovered in various plants and fungi,

although no single species contains more than 15 GAs (Phinney, 1979; Jones and Macmillan, 1984). They are synthesised in the apical leaf primordia, root tip and developing seeds. GAs act *in situ* in the organs producing them or are translocated to target organs where they evoke physiological response(s).

2.6.1 Physiological Roles of Gibberellins

The most prominent physiological effect of GAs is shoot elongation in intact plants. This response is clearly observed when GAs are applied to young plants. The growth promoting effect of GA_3 is caused by cell elongation and partially by cell division. Promotion of growth of various dwarf plants by gibberellins has been used as one common gibberellin bioassay (Reeve and Crozier, 1974; Crozier, 1981). Gibberellins have a unique ability among recognized phytohormones to promote extensive growth of many intact plants. Most dicots and some monocots respond by growing faster when treated with gibberellins, though some plant species show no response to GAs. Lack of response would probably mean that these species already contain sufficient amounts of GAs (Pharis and Kuo, 1977).

Dwarf mutants are often used as assay plants to estimate the physiological activity of GAs. Cabbages and other species in the rosette form that have short internodes sometimes grow 2 m tall and then flower after GA_3 application; the untreated controls remain short and vegetative (Salisbury and Ross, 1990). Such growth promoting effects have also been demonstrated in other experiments. Short beans becoming pole beans, genetic dwarf mutants of rice, maize, peas, watermelons, squash and cucumbers exhibiting phenotypically tall characteristics of normal varieties when treated with GA_3 or other GAs.

GA_3 was shown to significantly increase internode length, fresh weight and dry weight in *Pistiacia mutica* F.M. and *Pisticia khinjuk* stock seedlings (Baninasab and Rahemi, 1998). Shoot elongation and dry matter production were examined in rape (*Brassica napus*) seedlings grown under five light intensities (Potter *et al.*, 1999). Under low light, plants were tall, but produced little dry weight. As light intensity was increased, plants were progressively shorter, but had increasing dry weights. Endogenous GAs in stems of 16 and 17-day old plants were analysed by gas chromatography. The contents of GAs dramatically increased with decreasing light intensity. Shoot and hypocotyl lengths were closely positively correlated with (log) GA concentration, but shoot dry matter was negatively correlated with GA concentration. Application of GA_3 produced elongation of plants grown under high light, indicating that their low level of endogenous GA was limiting shoot elongation. These results indicated that GAs control shoot elongation, but are not directly involved in the regulation of shoot dry weight in rape. From these results it was further suggested that GAs have a role in photomorphogenesis, serving as an intermediate between light and the shoot elongation response (Potter *et al.*, 1999).

The effect of GA and NAA on hypocotyl elongation and cell wall polysaccharides was studied in *Phaseolus vulgaris* seedlings. GA was shown to promote hypocotyl growth, while NAA inhibited it. Xyloglucan content showed inverse correlation with growth. Pectic polysaccharides did not show a clear trend, though it showed an initial inverse correlation with growth. Results of this experiment led to the conclusion that degradation of low and high molecular weight xyloglucans are involved in cell wall loosening, which in turn may be responsible for the elongation of *Phaseolus vulgaris* hypocotyls in light (Bagatharia and Chanda, 1998).

In rice seedlings treatment with GA at 6.5 x 10^{-5} M was shown to positively affect cell elongation (Yim *et al.*, 1997). It also altered partitioning of carbohydrates. GA_3 induced more shoot growth and less accumulation of starch than the controls, but did not affect the photosynthetic ability (Yim *et al.*, 1997). Application of GA_3 at 100 ppm was shown to increase plant height of broad beans, *Vicia faba* (Abdul and Said, 1984).

Exogenous GA and auxins strongly stimulated stem elongation in dwarf GA-deficient mutants of light-grown peas. The auxins (IAA) stimulated growth by cell extension, while GA stimulated growth by an increase in cell elongation and number. It was noted that GA may enhance the auxin-induction of cell elongation but cannot promote elongation in the absence of auxins. The effect of GA may in part be mediated by auxins. Auxins and GA control separate processes that together contribute to stem elongation. It has therefore been suggested that a deficiency in either of the two harmones leads to a dwarfed phenotype (Yang Tao *et al.*, 1996). Gardner *et al.* (1985) reported that GA_3 stimulates internode elongation in plants. Pot-grown broad bean plants (*Vicia faba*) treated with 50 and 100 ppm GA_3 had increased height compared to the untreated control plants (Abdul and Said, 1984). Bean plants treated with 25, 50 or 100 ppm GA_3 showed increased stem length (El-fouly *et al.*, 1988). GA_3 at 50 and 100 ppm were shown to increase the leaf number and area in broad bean (Abdul and Said, 1984). In one month-old *Phaseolus vulgaris* cv. Carioca plants, GA_3 at 50 ppm was shown to increase plant height and the number of leaves per plant (Castro *et al.*, 1990).

Externally applied GA_3 was shown to enhance root elongation in peas cv. Alaska (Tanomoto and Barlow, 1995). Application of GA_3 was shown to reverse the inhibitory effects of ancymidol on root elongation. Ancymidol is an inhibitor of GA_3 biosynthesis.

Investigations on the effects of these PGRs on acid-induced elongation and cell wall components showed that GA_3 and ancymidol treated roots exhibited a rapid elongation response compared to ancymidol alone. Although GA_3 is known to increase the osmotic concentration of stem cells to enhance cell elongation; GA_3 did not increase the osmotic concentration of root cells. It is suggested that since cell elongation is regulated by cell wall extensibility and osmotic concentration for water uptake, GA_3-enhanced root elongation takes place due to a GA_3 effect on cell walls. GA_3-modified sugar composition, molecular mass of cell wall polysaccharides and the relative amounts of arabinose and galactose in the elongating cell walls of GA_3+ ancymidol treated roots was different from ancymidol-treated roots (Tanimoto and Barlow, 1995). GA_3 + ancymidol treated roots also contained longer molecular mass components of pectin and hemicellulose than ancymidol-treated roots. It is suggested that the GA_3 regulation and expansion of roots is accompanied by a modification of the matrix polysaccharides in the cell walls. Since ancymidol-treated roots were thicker than GA_3 + ancymidol-treated roots, these changes in the cell wall may reflect an increase in horizontal cell walls due to thickening (Tanimoto and Barlow, 1995).

Gibberellins promote wall plasticity, hence stem elongation and vegetative growth of the plants. Celery plants valued for the lengths and crispness of their stocks respond favourably to gibberellins, but the use of the GAs is limited due to the poor storability of such stalks (Salisbury and Ross, 1990). Certain growth retardants such as chlomequat and paclobutrazol inhibit stem elongation and cause overall stunting, because they inhibit gibberellin synthesis (Caldicolt and Lindely, 1964).

Many scientists have studied the mechanism by which gibberellins enhance cell elongation for a long time. There are varied theories that seek to explain the mode of action of GAs. A careful study by Liu and Loy (1976) showed that gibberellins promote cell division, because they stimulate cells in G_1 phase to enter the S phase and they also shorten the S phase.

Cell growth promotion by GAs sometimes results because they increase hydrolysis of starch, fructans and sucrose into glucose and fructose molecules. These hexoses provide energy via respiration and also contribute to cell wall formation. They also make the cell's water potential momentarily more negative; hence rapid entry of water causing cell expansion, but diluting the sugars (Salisbury and Ross, 1990). Gibberellins also cause increased synthesis of invertase enzymes that hydrolyze incoming sucrose into glucose and fructose in some species such as sugarcane (Glasziou, 1969).

Studies in lettuce hypocotyls have indicated that the ultimate action of GAs in this tissue is to bring about increased wall plasticity or to maintain the cell walls in a plastic state for a longer period (Stuart and Jones, 1977). The net result is a stimulation of elongation growth. In contrast to auxins, this change is produced without concomitant wall acidification or proton extrusion (Stuart and Jones, 1977). Adams (1975) showed that in the presence of sucrose and mineral salts to provide energy and prevent excessive dilution of cell contents, GA_3 treatment was shown to cause a 15-fold increase in elongation of young cells drawn from the intercalary meristem of oat internodes.

Recent studies have shown that hypocotyls fed with tritiated GA_1 accumulated significant amounts of labile in a purified cell wall fraction and this process preceded

growth response (Stoddart,1981). This suggests that the action of GAs may be related to the postponement of the formation of non-labile wall linkages, which ultimately preclude further extension growth. This is supported by the fact that ageing of hypocotyls progressively diminishes the response to a given dose of GA (Stoddart, 1981). If the primary action of GA is concerned with wall state, then the senescence delaying effect may be an expression of the maintenance of a sink for the photosynthate in the elongating tissues of the leaf. The effect could also result from the postponed deposition of secondary cell wall components, which limit the passage of metabolites between cells (Stoddart, 1981).

2.7 Effect of Plant Growth Regulators on Yield and Yield Components

In field beans (*Vicia faba* L.) GA_3 applied at 100 ppm was shown to increase pod set and the number of pods per plant (Abou-Elleil and El-Wazeki, 1978). In soybean, GA_3 application has been shown to increase the number of pods per branch (Bruce, 1990). In field beans, the number of fruit-bearing nodes and the number of pods per plant was shown to increase following GA_3 and BA application at the time of flowering (Nowak *et al.*, 1997). High level of GA_3 just before flowering was shown to lead to an increase in pod set, number of fruit-bearing nodes, pod weight and seed yield in field beans (Belluci *et al.*, 1982; Abdel-Fattah *et al.*, 1995).

Foliar sprays of TIBA (2,3,5-triiodobenzoic acid) at 50 or 100 ppm or naphthylphthalamic acid (NPA) to field beans at the beginning of flowering were shown to reduce shedding percentage and promoted pod setting, giving an increased final seed yield compared to the control. In treated pods, high levels of auxin and cytokinins were detected during pod development compared with untreated pods

(Shehata and Bondok, 1996).

Foliar application of 0.25 to 5.0 ml triacontanol/litre to French beans at three weeks after seedling emergence increased pod number per plant (Chikkasubbanna *et al.*, 1995). Nowak *et al.* (1997) reported that application of BA enhanced nitrogen concentration in seeds, pods and stems. Dybing and Westgate (1996) reported enhanced pod set in soybeans when cytokinins were applied directly to a flowering raceme.

In greenhouse trials on 1-month-old *Phaseolus. vulgaris* cv. Carioca plants, GA_3 at 50 ppm was shown to reduce pod weight and seed weight per plant (Castro *et al.*, 1990). In field trials on soybean cv. Forrest and Essex, GA_3 treatment increased the number of pods per branch. It also increased plant height, branch number and nodes per branch (Bruce, 1990). Application of BA at 1.0 mM directly to a flowering raceme of soybean was reported to enhance pod set, seed yield and flowers per raceme in some of the genotypes (Dybing and Westgate, 1996). In other genotypes however, it resulted in fewer seeds per pod, and smaller seed size on both treated and untreated racemes of the same plants (Dybing and Westgate, 1996)

Abdel-Fattah *et al.* (1995) reported reduced time to flowering in beans upon application of GA_3. Sarma and Shah (1981) earlier reported similar results in peas where GA_3 reduced the time to flowering by up to four days. Shahine *et al.* (1992) also reported that GA_3 application 30 and 45 days after sowing reduced the time to flowering and increased the number of flowers per plant in peas cv. Perfection.

Flower bud development in certain cultivars of French bean fail to produce pods in summer, because the flower buds do not develop normally and eventually drop off. This is attributed to the fact that flower bud development in these varieties is photoperiodic; it is inhibited by long days and promoted by short days (Morgan, 1981). Application of cytokinins at daily intervals to the developing shoot apex was reported to prevent flower bud abscission in long days (Morgan, 1981).

The effect of PGRs on pod yield may be attributed to their role on assimilate movement and partitioning within the plant system. PGRs have a significant role in the partitioning of photoassimilates. The mode of action of these hormones is considered to be related to the loading of assimilates at the source and to effects at the sink end of the phloem pathway (Patrick and Wareing, 1981).

Dry matter distribution significantly contributes to the yield potential of crop plants and would appear to largely account for the increased yields of cultivars over their ancestral stocks (Patrick and Wareing, 1981). Weaver and Johnson (1985) reported increased loading and unloading of assimilates across membrane boundaries of the vascular tissues of plants sprayed with cytokinins, leading to enhanced crop growth and dry matter production.

A number of recent studies have demonstrated a clear correlation between phytohormone content and the rate of dry matter accretion by the sink. For instance in the later phase of peach fruit growth where there is a substantial increase in dry matter accumulation, the rate of dry matter gain was found to parallel closely ethylene production by the fruit (Patrick and Wareing, 1981). Circumstantial evidence suggests

that assimilate partitioning is predominantly under sink control. The assembly of sink controls possibly includes hormonal signals emanating from the sink regions that act to regulate assimilate movement and distribution (Patrick and Wareing, 1981).

Excision experiments have yielded evidence that demonstrates the capacity of all classes of hormones to influence assimilate mobilization in several plant species. For example, replacement of the shoot apex of bean with IAA, GA_3 or kinetin resulted in an enhanced transfer of photosynthates to the hormone-treated stem stumps (Patrick and Wareing, 1981). The concept that phytohormones may have a role in determining the distribution of assimilates has been encouraged by the general observation that strong sinks for assimilates, such as growing fruits contain relatively high concentrations of plant growth substances (Patrick and Wareing, 1981).

Assimilate movement can be partially restored in peduncles by treatment with PGRs following either or fruit removal flower (Patrick and Wareing, 1981). It can be concluded that there is a strong, but predominantly circumstantial evidence for phytohormone participation in assimilate partitioning. Plant growth regulators have the ability to affect metabolite uptake independent of a growth response. Plant growth regulators therefore have a potential to influence assimilate movement through their action on either sink activity and/or sink size. Rate of assimilate transfer from the phloem (and hence the sink's competitive ability) could be modulated by PGRs influencing phloem unloading and/or sink uptake processes (Patrick and Wareing, 1981).

The fact that hormone effects are restricted to the site of application tends to support the view that the hormone acts by promoting sink uptake (Patrick and Wareing, 1981). The 'green islands' effect of cytokinins reported by (Morgan, 1981) supports the fact that PGRs like cytokinins increase sink uptake of assimilates.

2.8 The Role of Plant Growth Regulators on Chlorophyll Synthesis, Retention and Senescence in Plant Tissues

Senescence refers to those changes that provide for the endogenous regulation of death (Leopold *et al.*, 1959). It can also be defined as the series of events concerned with the cellular disassembly of the plant tissue and the mobilization of materials released during this process (Stoddart, 1981). Salisbury and Ross (1990) define senescence as the processes of deterioration that accompany aging and lead to death of an organ or organism. Just before termination of a leaf's effective life there is an abrupt and rapid deterioration of its chlorophyll content and a change in colour from green to yellow. This chlorophyll decline makes an easy and convenient measure of the progress of leaf senescence. For this reason leaves are widely used in senescence studies (Leopold, 1959).

Leaf senescence is accompanied by early losses in chlorophyll, RNA and proteins including many enzymes. These losses could be a result of slower synthesis and/or faster breakdown of these macromolecules (Salisbury and Ross, 1990). Humbeck *et al.* (1996) reported that during senescence, chlorophyll and photosynthetic proteins in leaves are degraded; therefore along with chlorophyll disappearance goes the process of photosynthesis. Exogenous application of cytokinins has been shown to inhibit the

degradation of chlorophyll and photosynthetic proteins (Richmond and Lang. 1957; Badenoch-Jones *et al.*, 1996). Van Staden and Joughin (1988) reported that cytokinins enhance greater retention of chlorophyll and protein content. Sacher (1973) reported that cytokinins maintain a high protein content by retarding the rate of breakdown rather than enhancing the rate of synthesis.

Evidence that cytokinins can control senescence has mainly come from studies employing three approaches namely: a) External application of cytokinin; sometimes known as the 'spray and pray' approach. These studies provided the initial evidence that cytokinins play a role in senescence. b) Measurement of endogenous levels of cytokinins during senescence; these studies revealed an inverse correlation between cytokinin levels and senescence progression. c) The most recently developed is the transgenic plant strategy that employs the expression of an enzyme catalysing the limiting step in cytokinin biosynthesis to elevate the endogenous cytokinin levels (Gaan and Amasino, 1995).

Cytokinin treatment experiments demonstrate that cytokinins can interfere with senescence in detached tissues of dicotyledons and monocotyledons, but the effects are less effective in attached tissues (Van Staden *et al.*, 1988). Richmond and Lang (1957) reported that exogenous application of cytokinins to detached cocklebur leaves inhibited the degradation of chlorophyll and photosynthetic proteins. In excised oat leaves, cytokinins were reported to retard the breakdown of chlorophyll and protein and delay the onset of rising respiration associated with leaf senescence (Thimann, 1980). Externally applied cytokinins have been reported to cause yellowing leaves to regreen (Dyer and Osborne, 1971). Salisbury and Ross (1990) reported the ability of cytokinins

to enhance subsequent development of etioplasts into chloroplasts and increase the rate of chlorophyll formation. This could be because cytokinins enhance formation of one or more proteins to which chlorophyll binds and becomes stabilized (Salisbury and Ross, 1990).

Beyer, (1981) reported that the degreening effects of ethylene on citrus fruits were through the *de novo* synthesis of chlorophyllase protein and the increase of chlorophyllase enzyme activity. These effects of ethylene were in turn inhibited by the senescence delaying PGRs, GA and N^6-benzyladenine. Benzyladenine and GA inhibited *de novo* synthesis of the enzyme that is involved in the degradation of chlorophyll (Beyer, 1981).

Other organisms that interact with plants have taken advantage of the ability of applied cytokinins to affect senescence. A number of pathogens are able to maintain greenness and full photosynthetic function in a radial zone around the infection site (Stoddart, 1981). These pathogens secrete cytokinin-like substances that diffuse in and cause greening. Similar observations have been reported by (Morgan 1981). He noted that certain insects' larvae and phytopathogens e.g. powdery mildew (*Erysiphe graminis*) and rust fungi (e.g *Uromyces phaseoli*) could synthesize cytokinins. The produced cytokinins are then applied to plant tissues surrounding sites of infection to prevent host tissues cells from dying thereby forming regions known as 'green islands'. Spot application of kinetin solution produces similar "green islands" (Morgan, 1981). These observations support the concept of hormone-directed transport which suggests that the preferential accumulation of transported metabolites in zones where spot applications of cytokinins have been made is due to the metabolism-directing properties of the growth

regulator (Stoddart, 1981). It can be concluded that the important factor is the high rate of metabolism in such areas, resulting from the delayed senescence.

The senescence delaying ability of cytokinins has been explored in some horticultural commodities. The storage lives of brussel sprouts and celery are often increased by the relatively inexpensive commercial cytokinins such as benzyladenine (Salisbury and Ross, 1990). In broccoli postharvest treatment of florets with BA at 50 ppm was reported to have pronounced effects compared to the controls (Rushing, 1990). The rate of respiration was reduced by 50%, and ethylene production increased by 40% throughout the first 4 days of storage. Total chlorophyll content dropped by 40% throughout the first 4 days of storage. Total chlorophyll content dropped by 60% in controls, but was unchanged in cytokinin-treated florets, which had a 90% longer shelf-life than controls (Rushing, 1990).

Van Staden and Joughin (1988) reported that cut-flower longevity could be increased as a result of senescence retarding effects of cytokinins. In an experiment to determine the effects of benzyladenine, gibberellic acid, naphthalene acetic acid and maleic hydrazide at 50 or 100 ppm on the vase-life of tuberose spikes, Bhaskar and Rao (1998) showed that BA and GA_3 improved water uptake and maintained a better water balance. This, in turn increased the vase-life and the number of florets, which opened per spike. Addition of 100 ppm BA to the vase water delayed senescence and maintained freshness for a longer time than a similar concentration of GA_3 (Bhaskar and Rao, 1998).

Zeatin riboside and 6-Benzylaminopurine, the principal cytokinins in corollas at the time of corolla opening, were shown to be effective senescence retardants in Petunia

corolla (Taverner *et al.*, 1999). O-glucosyldihydrozeatin riboside was considerably less effective. Corolla senescence was preceded by a rise in cytokinin O-glucoside level, O-glucosyldihydrozeatin being the principal glucoside formed (Taverner *et al.*, 1999). Exogenous ethylene promoted conversion of dihydrozeatin to O-glucosides, but not 7-glucoside. Ethylene also promoted the conversion of zeatin riboside to adenosine and adenosine monophosphate. Hence it appears that ethylene production, which induces corolla senescence, also promotes inactivation of cytokinins by O-glucosylation and degradation, and this may facilitate the senescence process (Taverner *et al.*, 1999).

In cut chrysanthemum, BA treatment delayed senescence and also improved vase-life and quality by improving membrane stability. Membrane stability was improved by reducing membrane permeability, delaying the peroxidation of membrane lipids, reducing the production of malondialdehyde, and altering the activities of peroxidase and superoxide dismutase compared with control (Zhang *et al.*, 1998). Mutui (1999) reported that BA delayed the degradation of chlorophyll by delaying the breakdown of protein used in chlorophyll synthesis. This was evidenced by high retention of nitrogen in leaves of BA-treated cut Alstromeria flowers (Mutui, 1999).

In potted Asiflorum lily cv. Donau, application of 0, 50, 250, or 500 mg/litre BA equivalent of Promalin (GA_{4+7}:BA ratio of 1:1) or Accel (GA_{4+7}:BA ratio of 1:10) delayed leaf senescence (Funnell and Heins, 1998). The delay increased with increasing concentrations of either Promalin or Accel, but Promalin was more effective. Application of Promalin at 250 mg/litre BA equivalent completely eliminated leaf senescence over the evaluation period. The treatments did not affect flower bud opening or plant height (Funnell and Heins, 1998)

Premature leaf yellowing is a physiological disorder of melons occurring during fruit maturation (2 to 3 weeks preharvest). It is common in autumn crops in the Lower Jordan Valley (Nerson *et al.*, 1988). Treatments of muskmelons cv. Galia with BA at 10 ppm and foliar nutrients applied weekly or fortnightly as sprays between flowering and fruit harvest were effective in preventing early leaf yellowing. There was also a positive correlation between the ability of BA to maintain green foliage and the sweetness of the fruits (Nerson *et al.*, 1988).

Root tips are the major source of cytokinin supply in intact plants (Richmond and Lang, 1957). Leaf senescence seems to be controlled by the supply of cytokinins from the root system. This explains the rapid yellowing of detached plant tissues such as leaves and the ineffectiveness of cytokinins in delaying senescence in attached tissues (Richmond and Lang, 1957). Yellowing of excised leaves is postponed or eliminated when roots are initiated on the cuttings. It has been shown in xanthium that exogenous cytokinin can substitute for roots in this respect (Richmond and Lang, 1957).

Cytokinins promote grana formation and increase the rate of chlorophyll formation (Lew and Tsuji, 1982). They activate the synthesis of two proteins of the chloroplast; RUBP carboxylase and the chlorophyll a/b protein complex (Funkees-Shippy and Levine, 1985). BA was reported to induce increases in chlorophyll content per chloroplast, chloroplast DNA content and fresh weight per leaf in *Phaseolus vulgaris* leaves (Momotani *et al.*, 1991).

At cellular level, changes in the protein profile and cell integrity have been used as pointers to senescence (Richmond *et al.*, 1970). Ribonucleic Acid (RNA) has been shown to decline during senescence, roughly in parallel with the decline in protein and DNA. At the same time RNA continues to be synthesized; the synthesis may be both from adenine and kinetin (Richmond *et al.*, 1970). Reported that cytokinin treatment could result in maintenance of RNA and soluble protein levels, stabilization of polysome aggregate and the suppression of changes in respiration rate and the mitochondrial coupling, normally associated with senescence. This response is generally confined to excised tissues and only very small effects are evident in attached tissues like leaves (Stoddart, 1981).

During maturation and senescence, disappearance of the ribosomes was observed; first of free, single ribosomes, then of those aggregated into clusters and finally of those attached to the endoplasmic reticulum, which vesiculates (Butler and Simon, 1971). Two major metabolic events occurring in senescing petals are, an increase in respiration and hydrolysis of cell components. Enzymic changes found during petal senescence are associated mainly with these two processes. An increase in peroxidases was found in senescing petals of several plants (Brendmeijer, 1973; Carfantan and Daussany, 1975). In an investigation to determine the influence of BA on peroxidase activity during the senescence of sunflower cotyledon, the level of total chlorophyll was used as an indicator of senescence. As senescence progressed, peroxidase activity in the cotyledons increased, while the level of total chlorophyll decreased. In the BA-treated cotyledons, the activity of peroxidase and the number of peroxidase isoenzymes decreased compared with the control, whereas the level of total chlorophyll was higher than the control (Durmus and Kadioglu, 1998). The increased activity of peroxidases is

apparently related to an increase in peroxides and free radicals, which react with cellular constituents (Fridovich, 1975) involved in promotion of senescence (Brennan and Frenkel, 1977; Mishra *et al.*, 1976) and possibly also in ethylene production (Beauchamp and Fridovitch, 1970).

During the course of petal aging there is a drop in the level of macromolecular components such as starch (Ho and Nichols, 1977), cell wall polysaccharides (Wiemken *et al.*, 1974), proteins (Borochov *et al.*, 1976; Parups, 1971; Paulin, 1971) and nucleic acids. Petal senescence is also accompanied by a decrease in RNA, an increase in RNnase activity and degradation of DNA. It was also shown that hydrolytic enzymes including RNase, β-glycosidase and b-galactosidase were synthesised *de novo*, while the total protein content of the corolla declined (Baumgartner *et al.*, 1975; Wagner and Siegelman, 1975). This indicates the requirement of protein for the onset of senescence (Arditti and Knauft 1969; Dilley and Carpenter, 1975).

The decline in protein and nucleic acid levels during plant senescence (Hardwick and Woolhouse, 1967; Hurst and Gahan, 1975; Woolhouse, 1967) is perhaps the most basic of all senescence related events. Protein, RNA and DNA of senescent tissues all decline but at different rates, that of DNA being the least (Callow, 1974; Callow *et al.*, 1972; Hardwick and Woolhouse, 1967). The initial decline of protein and nucleic acid components in the chloroplast involves a decrease in the major chloroplast enzyme RUBPCase (Kannangara and Woolhouse, 1967; Callow, 1974; Kawashima *et al.*, 1967) along with other enzymes known to be synthesized at least in part on chloroplast ribosomes (Batt and Woolhouse, 1975; Spencer and Titus, 1972). The loss of these enzymes correlates with a reduction in the number of chloroplast polysomes (Callow *et*

al., 1972), a decline of chloroplast RNA levels (Callow *et al.*, 1972; Callow, 1974; Takegami, 1975) and a major reduction in the ability of the chloroplast to synthesize proteins and RNA (Hardy *et al.*, 1968; Paranjothy and Wareing, 1971; Spencer, 1973; Takegami, 1975). The gradual decline is followed by a period of more rapid plant senescence. This period is usually marked by the initiation of yellowing (chlorosis) and involves many new changes in protein and nucleic acid metabolism. During the rapid decline, there is accelerated protein and nucleic acid degradation in both the cytoplasm and the chloroplast along the decrease in synthetic capacity of the whole cell (Batt and Woolhouse, 1975; Callow *et al.*, 1972; Hardwick and Woolhouse, 1967; Kannangara and Woolhouse; 1967; Wollgiehn, 1967; Woolhouse, 1978).

Studies on the correlation between cytokinin levels and senescence progression through the analysis of cytokinin levels during plant development have revealed an inverse correlation between cytokinin levels and progression of senescence in a variety of tissues and plant species (Van Staden *et al.*, 1988). In soybean plants, xylem sap cytokinin levels drop sharply with the onset of leaf senescence.

In the transgenic plant strategy, the cloning of a gene encoding a cytokinin–synthesizing enzyme has provided a way to genetically engineer plants (Kaminek, 1992). In these genetically engineered plants, the endogenous cytokinin levels can be manipulated. This transgenic approach complements the conventional approaches and provides confirmation of the regulatory effects of cytokinins in senescence. Although some cytokinins could be provided by tRNA breakdown, it is generally accepted that the primary biosynthetic pathway begins with the transfer of an isopentyl group from Δ^2 -isopentyl pyrophosphate to adenosine 5'-phosphate (AMP) to form isopentenyladenine ribotide. The isopentenyladenine ribotide is then converted to various forms. The first

committed step is catalyzed by isopentenyl transferase or iptase, which is also called cytokinin synthetase (Kaminek, 1992). A bacterial version of isopentenyl transferase encoded by the *IPT* gene has been identified in studies of tumor formation caused by the Ti (tumor-inducing) plasmid of *Agrobacterium tumefasciens* (Akiyoshi *et al*, 1984; Barry *et al* 1984). In the plant cell, expression of *IPT* and genes involved in the synthesis of the auxin class of plant hormones causes overproduction of cytokinins and auxins. In plant cells, the level of isopentyl transferase is the limiting factor in cytokinin biosynthesis, because expression of *IPT* alone can cause overproduction of cytokinins (Kaminek, 1992). In several studies, the elevated endogenous cytokinin levels in transgenic plants that express *IPT* delay senescence, especially leaf senescence (Smart *et al.*, 1991; Gan and Amasino, 1995).

In most studies, increased chlorophyll synthesis or retention is attributed to cytokinin effects. However, gibberellins have also been shown to contribute to chlorophyll synthesis and retention. They interfere with the degradation of chlorophyll as well as with the biosynthesis of carotenoids and anthocyaninins (Dostal and Leopold, 1967). Concentrations as low as 0.1 mg /litre of GA_3 effectively delay degreening of detached citrus fruits and when sprayed on the tree, the effects lasted for several months Gibberelins were reported to enhance the regreening of Valencia oranges. Indicating that gibberellins act as inducers of green chloropast development and not merely as inhibitors of senescence (Goldschmidt, 1973).

Leaf senescence was delayed in Alstromeria by application of various gibberellins and cytokinins (Jordi *et al.*, 1995). Some gibberellins (GA_4 and GA_7) were far more

effective in delaying chlorophyll loss than GA_3, which is commonly used as a postharvest treatment for alstromeria cut-flowers (Jordi *et al.*, 1995).

2.9 Effects of Plant Growth Regulators on Postharvest Water Loss and Retention

One of the most important factors determining the postharvest shelf-life of horticultural commodities is their ability to maintain turgidity. A high level of turgidity is necessary for the continuance of normal metabolic activities because the commodities continue living even after being detached from the mother plant (Rogers, 1973). Symptoms of the final stages of senescence in petals are loss of fresh weight, drying and shriveling. Plant growth regulators are known to have a significant effect on water balance in plant tissues. Gibberellins have been known to enhance water retention in plant cells, hence increase fresh weight (Salisbury and Ross, 1990). Because water retention, hence turgidity is important in postharvest shelf-life of fresh produce, GAs could play a significant role in enhancing postharvest shelf-life of perishable commodities.

In cut-flowers, water relations are influenced to a great deal by PGRs (Mayak and Halevy, 1974). In an experiment to determine the effects of benzyladenine, gibberellic acid, NAA and maleic hydrazide (at concentrations of 50 or 100 ppm) on the vase-life of tuberose spikes; addition of BA and GA_3 at 50 or 100 ppm to the vase solution was reported to improve water uptake and maintain a better water balance. This in turn increased the vase-life and the number of florets that opened per spike. Benzyladenine at 100 ppm delayed senescence and maintained freshness for a longer time than a similar concentration of GA_3 (Bhaskar and Rao, 1998).

In some cut-flowers, water loss from aging petals occurs even when they are held in water. In such flowers an infiltration of the intracellular spaces is apparent (Horie, 1961). This suggests that there may have been a loss of membrane integrity, causing an increase in permeability and leakage. An increase in apparent free space has been recorded in aging spadix of Arum (Eialm, 1965) and rose petals (Parups and Chan, 1973). An increase in membrane permeability was demonstrated during senescence of several flowers (Hanson and Kende, 1975; Mayak *et al.*, 1977; Nicholas, 1968). A sharp increase in microviscosity of the plasmalemma was observed during aging. This corresponded to an increase in the mole ratio of free sterol to phospholipid. The increase was attributed to a rise in the activity of phospholipase, which brought about a decrease in the level of phospholipids. The decrease in phospholipids should enhance the permeability of the plasma membrane and make it leaky (Simon, 1974). Cytokinin/ABA balance was suggested to control membrane modification (Itai and Benzioni, 1976). Kinetin was shown to delay wilting of cut-rose flowers by protecting cell integrity (Mayak and Halevy, 1974).

Treatment of bean seeds with GA_3 and ethephon at 100 ppm resulted in increased water uptake, respiration rate, water retention capacity and catalase activity. The treatments also resulted in higher total soluble solutes (TSS), sucrose and soluble protein contents in the cotyledons compared with the controls (Tao – HanZhi *et al.*, 1995). GA is known to increase the osmotic concentration of cells, resulting in enhanced water uptake that leads to cell elongation (Salisbury and Ross, 1990).

2.10 Effects of Plant Growth Regulators on Chilling Injury and Some Postharvest Qualities of Fruits and Vegetables

Some tropical and subtropical fruits and vegetables suffer chilling injury when exposed to low (non-freezing) temperatures. The symptoms of such injuries vary among species. They usually include staining of the peel and internal browning, and are related to modifications at the cell membrane level. Susceptibility to low temperatures varies in different species, and different mechanisms of damage and of natural resistance are known (Douglas and Paleg, 1974; Douglas and Paleg, 1981; Abeles, 1973; Goodwin, 1978).

It is necessary to find ways of increasing plant resistance and tolerance to low temperatures; this will help alleviate chilling injury-related losses. Besides breeding resistant genotypes, attempts have been made to enhance low temperature resistance by chemical treatment. Some plant growth regulators are known to reduce the chilling injury. Polyamines (putrescine, spermidine and spermine) have an antisenescent action, because of their capacity to link with anionic compounds in the cell membrane and to capture free radicals, thus stabilizing the lipid bilayer and preventing membrane deterioration (Serrano, 1996).

Chlormequat (CCC) has been used in treating seed to increase winter resistance of wheat seedlings (Wunsche, 1981; Roberts, 1971). Besides CCC, other compounds such as 2-amino-6-methyl benzoic acid and long-chained alkalyne diamines have been found to protect many cereals from frost damage (De Silva *et al.*, 1979; Okii, 1980).

In a study to determine the effects of postharvest treatments on weight loss, decay, chilling injury and keeping quality of Balady oranges, treatment with wax emulsion and/or GA_3 resulted in the highest values for total soluble solids (TSS) and keeping quality (Attia, 1995).

There is a need to explore the use of other PGRs such cytokinins, gibberellins and auxins to reduce the negative effects of low temperatures and alleviate chilling injury-related losses in food crops.

2.11 Other Effects of GAs on Plant Growth and Development

Gibberellins overcome seed and bud dormancy in many species. They thus act as a substitute of low temperatures, long days and red light requirement in these species to break dormancy. Gibberellins are required for the germination of seeds (Gardener *et al.*, 1985). In seeds, the principal gibberellin effect is to enhance cell elongation so that the radicle can push through the endosperm, seedcoat or fruit that restricts growth. Gibberellins also replace the requirement for vernalization in biennals (Salisbury and Ross, 1990; Wittwer, 1983).

In some species, GAs promote parthenocarpic fruit development (Halman, 1990), suggesting a normal function in fruit growth. Gibberellins also stimulate the mobilisation of food and minerals in seed storage cells, especially in cereal grains (Akazawa and Miyata, 1982).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Site

Two field experiments were carried out in the 1998 and 1999 growing seasons. The first trial was performed between November 1998 and January 1999. The second trial was between February 1999 to April 1999. The experiments were carried out at the Field Station Farm of Kabete campus, Faculty of Agriculture, University of Nairobi. This site lies at an altitude of 1940 m above sea level and lies at latitudes 1^0 14' 20" to 1^0 15' 15" South and longitudes 36° 44' to 36° 45' East. The mean monthly maximum temperature is 23°C, while the mean minimum temperature is 12°C. The area has a bimodal rainfall pattern, with peaks in April and November. The annual rainfall is slightly above 1000 mm. The soils in Kabete have been taxonomically described as humic nitosols peleustuist (Siderius, 1976). They are deep friable kaolinitic clay types formed *in situ* from the tertiary trachytic lava (Siderius, 1976).

3.2 Treatments and Experiment Design

The treatments consisted of different levels of plant growth regulators (PGRs) namely Accel® and Provide® (trade names). Provide is a liquid concentrate containing 21 g a.i/litre (w/w) GA_{4+7} (Abbott Laboratories, North Chicago, USA). Accel is a liquid concentrate containing 20 g a.i/litre (w/w) 6-benzyladenine (BA) and 2 g a.i/litre gibberellins; GA_{4+7} (Abbott Laboratories, North Chicago, USA). Accel was applied at 0, 12.5, 25.0, 37.5 and 50.0 mg/litre BA equivalent. Provide was applied at 0, 1.25, 2.50, 3.75 and 5.0 mg/litre to give equivalent amount of GA_{4+7} when, Accel is applied

to the bean plants. Effects of BA were therefore determined by subtracting the values obtained due to GA_{4+7} effects from those of Accel for each variable.

In both seasons, the experiment was laid down as a randomized complete block design with the treatments randomly arranged in each block. There were four blocks in each of the experiments. In each block, nine experimental plots, measuring 3 m x 3 m each were mapped out and furrows 0.4 m apart, were then cut across the gradient. Diiammonium Phosphate at a rate of 200 Kg/ha was then evenly spread into the furrows and thoroughly mixed with the soil therein. The beans were then hand placed along the furrow at a spacing of 10 cm between plants and lightly covered with soil. In the second experiment, Furadan was topdressed to prevent the damage by cutworms. In both seasons 30 ml dimethoate per 20 litres of water was sprayed to control aphids.

In both experiments, hand weeding was done twice during the growing season; the first weeding was done two weeks after emergence and the second one just before flowering. In the two experiments French bean seeds of cultivar Monel were used. The plant growth regulators were sprayed to run-off using a hand sprayer, four weeks after emergence.

3.3 Data Collection

Two outer rows of each experimental plot were treated as guard rows. In each plot, ten plants from two central rows were tagged for yield and yield components determination. The rest of the plants were used to collect data on the other variables.

3.3.1 Pod length

A cumulative total of 40 pods harvested from each of the 10 tagged plants in every exprimental plot were used to determine the average pod length. In each case the pods harvested from each plant were arrayed on a clean table and a ruler was used to measure the length of the pods in centimetres. The average length of pods was computed for each of the 10 plants.

3.3.2 Pod number

Harvesting was done 10 times during the growing season. In each harvest, pods from the 10 tagged plants were harvested and counted. At the end of harvesting the cumulative number of pods per plant was determined and the average number of pods per plant determined.

3.3.3 Pod yield

For each harvest pods from each of the 10 tagged plants were harvested separately into paper bags and carried to the weighing room immediately to avoid water loss. The pods were then removed from the paper bags and weighed separately on a top pan balance to determine the fresh weight. The cumulative weight of pods from the 10 harvests was computed for each plant. From this, the total weight of pods harvested from the 10 plants in each plot (experimental unit) was computed and the mean thereof determined.

3.3.4 Water content of pods

Forty pods per plot were picked randomly from the bulk harvest and their fresh weight determined. The 40 pods were then packed into brown paper bags and oven-dried at 66 °C to a constant weight. Dried pod samples were then weighed for dry matter

determination. The dry weight was subtracted from the fresh weight to determine the water content of the pods. Percentage water content was computed using the formula:

$$\text{Water content \%} = \frac{(\text{Fresh weight} - \text{Dry weight})}{\text{Fresh weight}} * 100$$

3.3.5 Chlorophyll content determination

3.3.5.1 Leaf chlorophyll content

Ten leaves were picked randomly from each plot (experimental unit) and from each leaf 1 disc of 1 cm diameter was obtained using a 1 cm cork borer. A total of 10 discs bored from 10 leaves were then extracted in 4 ml of 0.1N HCl in 88% Methanol. The extraction was done at 21°C in a dark room for 24 hours. The absorbance of the extracts was measured using a Spectrophotometer, WPA S105. The chlorophyll content was measured as absorbance of the extracts at 653 nm (Holden, 1965; Douglas, 1983). The equation below was used to calculate the relative total chlorophyll content per unit leaf area (Douglas, 1983).

$$\text{Chlorophyll } (mg/cm^2 \text{ leaf}) = 24.88 \times A_{653nm}$$

where: A is the absorbance at 653 nm and 24.88 is a molar extinction coefficient.

3.3.5.2 Pod chlorophyll content

Pod chlorophyll content was determined from 10 slices each of area 0.785 cm^2. The slices were obtained using a scapel blade. The thin slices of negligible thickness (as thick as the leaves) were obtained from the outer layer of 10 pods selected randomly from each treatment. The 10 slices were extracted in 4 ml of 0.1N HCl in Methanol

(88%). Extraction proceeded for 24 hours at 21°C in a dark room. The absorbance of the extracts was measured using a spectrophotometer. The chlorophyll content per unit pod area waas calculated using Douglas (1983) formula as shown above (under 3.3.5.1).

3.3.5.3 Chlorophyll retention

Some of the pods were stored at ambient room temperature fluctuating between 18°C - 22°C. The relative humidity (RH) of ambient air averaged 70.6% and 76.5% in the first season and second season, respectively. Cholorophyll retention by the pods after 8 days of storage was determined using the procedure described in section 3.3.5.1 above.

3.3.6 Postharvest storage

Pods harvested from each of the plots (experimental unit) were divided into 2 batches to be stored under different conditions. One batch was stored at ambient room temperature fluctuating between 18°C to 22°C. The relative humidity (RH) of ambient air averaged 70.6% and 76.5% in the first season and second season respectively. The second batch was stored in a refrigerator at a temperature of 4±0.5°C and RH of 98%.

Pods were packed into nylon paper bags, which are the common packaging material for French beans. The following dependent variables were determined:

i) weight loss by the pods

ii) the degree of wilting

iii) chlorophyll retention after storage

Samples in the refrigerated storage were examined for chilling injury symptoms (namely browning and surface pitting of pods) and rate of water loss after 21 days of cold storage.

3.3.6.1 Water loss

Forty pods were randomly selected from a sample harvested from each of the experimental units. The initial weight was accurately determined using a top pan balance. The pods were then stored under ambient room conditions. The pods were weighed every day to determine the daily water loss for the subsequent 7 days of storage. For each day of storage, water loss was determined as the weight of pods on the given day subtracted from the initial weight. For pods that had been stored in the cold room, the water loss at ambient room temperature and RH was determined after 21 days of cold storage at $4\pm0.5^{o}C$.

3.3.6.2 Determination of wilting

This was done by carrying out a snapping test on a sample of forty pods after storage at ambient room conditions. Each pod at a time was held so as to leave a space of about 8 cm between fingers. The pod was then bent to form a closed loop, if the pod looped without snapping it was considered wilted. If the pod snapped before making a loop, it was considered still turgid and good (Njeru, 1989). The number of pods that looped out was expressed as a percentage of the initial number of pods (forty) used to do the test.

3.3.6.3 Determination of percent chilling injury

From each experimental unit a random sample of forty pods were selected, cleaned and packaged into nylon paper bags. The pods were then stored in the refrigerator at a temperature of 4±0.5°C for 21 days. After the 21 days of storage, pods from each package were visually examined for symptoms of chilling injury. These symptoms included surface pitting and browning of pods. The number of pods showing chilling injury symptoms were expressed as a percentage of the initial 40 pods.

After the 21 days of cold storage, pods were removed from the fridge and exposed to ambient air conditions for evaluation of post storage stability. This was established by determining the rate of water loss after cold storage. The initial weight of the pods after cold storage was determined and daily water loss determined by subtracting the weight of the pods on a given day from the initial weight.

3.3.7 Data analysis

Analysis of variance was performed on the data collected using the general linear models (Proc GLM) procedure of the Statistical Analysis System (SAS) programme. Linear, quadratic and cubic orthogonal polynomials were tested and appropriate regression models were used to determine the nature of the response to benzyladenine (BA) and gibberellins (GA) concentration (Snedecor and Cochran, 1989). Multiple comparisons among means were performed using the protected Least Significant Difference (LSD) at P=0.05. Proc Univariate procedure was carried out on the residuals to support the assumptions of normality made by the researcher.

CHAPTER FOUR

RESULTS

4.1 Effect of GA_{4+7} on Pod Length, Pod Number and Fresh Pod Yield

4.1.1 Pod length

Application of GA_{4+7} significantly increased pod length (Table 1). In both experiments 1 and 2, the increases in pod length effected by GA_{4+7} at 1.25, 2.5, 3.75 or 5.0 mg/litre were not significantly different from each other. Thus the effect was not dependent on the concentration of GA_{4+7}.

4.1.2 Number of pods

Application of GA_{4+7} significantly increased the number of pods per plant in both experiments, except GA_{4+7} at 1.25 mg/litre, which had no significant effect in experiment 1 (Table 1). In both experimente the effects of GA_{4+7} at 25.0, 37.5 or 50.0 mg/litre on pod number were not significantly different from each other (Table 1).

4.1.3 Pod yield

Application of GA_{4+7} increased pod yield compared to the control in both experiments 1 and 2 (Table 1). In experiment 1, GA_{4+7} at 1.25, 2.5, 3.75 and 5.0 mg/litre all increased pod yield with level 3.75 mg/litre resulting in a significantly higher yield than the other GA_{4+7} concentrations (Table 1). In experiment 2, GA_{4+7} at 2.5, 3.75 and 5.0 mg/litre were not different in their effect on pod yield (Table 1). GA_{4+7} at 1.25 mg/litre increased pod yield but to a lesser extend compared to levels 2.5, 3.75 and 5.0 mg/litre (Table 1).

Table 1. Effect of GA_{4+7} on pod length, pod number and pod yield of 'Monel' French beans.

FIRST EXEPERIMENT				SECOND EXPERIMENT		
GA_{4+7} (mg/l)	Pod length (cm)	Pod number	Yield (tons/ha)	Pod length (cm)	Pod number	Yield (tons/ha)
0	10.72b	56.18b	35.57d	10.77b	55.78c	37.62c
1.25	11.12a	58.23b	36.88c	11.14a	58.33b	39.58b
2.5	11.05a	65.45a	40.25b	11.36a	63.05a	41.20a
3.75	11.29a	66.33a	41.52a	11.29a	64.33a	41.28a
5.0	11.28a	63.13a	40.21b	11.35a	63.45a	41.43a
Significance	**	****	****	**	****	****
LSD	0.25	3.99	1.02	0.32	2.14	1.34

,*,****, significance within columns at P= 0.01, 0.001 and 0.0001, respectively.

M ans were separated by the protected LSD (P=0.05); Means with the same letter(s) within columns are not significantly different.

4.2 Effect of Accel on Pod Length, Pod Number per Plant and Fresh Pod Yield

4.2.1 Pod length

Application of Accel at 12.5, 25.0, 37.5 or 50.0 mg/litre BA equivalent significantly increased pod length in both experiments 1 and 2 (Table 2). In exeperiment 2, there were no significant differences among Accel concentrations with respect to their effect on pod length, but the response to increasing Accel concentration was quadratic (Table 2). In experiment 1, the response was neither linear nor quadratic.

4.2.2 Number of pods

Application of Accel increased the number of pods per plant in both experiments 1 and 2 (Table 2). In experiment 1, Accel at 37.5 mg/litre BA equivalent increased pod number per plant more than at to 12.5, 25.0 and 50.0 mg/litre BA equivalent (Table 2). However, Accel at 37.5 mg/litre BA equivalent was not significantly different from 50.0 mg/litre BA equivalent on their effect on pod number per plant (Table 2). In experiment 2, application of Accel to French beans at 12.5, 25.0, 37.5 and 50.0 mg/litre BA equivalent increased pod number per plant compared to the control. The response to increasing Accel concentration was linear. There were no significant differences among levels 25.0, 37.5 and 50.0 mg/litre of BA equivalent with respect to their effects on the number of pods per plant (Table 2).

4.2.3 Fresh pod yield

Application of Accel significantly increased pod yield of French beans in both experiments 1 and 2 (Table 2). In experiment 1, Accel at 25.0, 37.5 and 50.0 mg/litre BA equivalent increased pod yield and there was no significant difference between 25.0

Table 2. Effect of Accel on Pod Length, Pod Number per Plant and Pod Yield of 'Monel' French Beans.

FIRST EXEPERIMENT				SECOND EXPERIMENT		
ACCEL mg/l	**Pod length (cm)**	**Pod number**	**Yield (tons/ha)**	**Pod length (cm)**	**Pod number**	**Yield (tons/ha)**
0	10.72b	56.18d	35.57c	10.77b	55.78c	37.64c
12.5	10.94ab	60.73c	37.12c	11.29a	59.95b	40.56b
25.0	11.17a	64.10bc	41.22b	11.52a	62.68a	41.98ba
37.5	10.99ab	67.60a	44.04a	11.43a	63.88a	42.39a
50.0	11.28a	65.43ba	41.64b	11.35a	63.85a	41.63ba
Significance[z]	**	****	C****	Q***	L****	L****
LSD	0.34	3.44	1.56	0.29	2.41	1.55

[z] the response was linear (L), quadratic (Q) or cubic (C)

, *, ****, significance within columns at P=0.01, 0.001 and 0.0001 respectively.

Means were separated by the protected LSD (P=0.05); Means with the same letter(s) within columns are not significantly different.

and 50.0 mg/litre BA equivalent (Table 2). Accel at 37.5 mg/litre BA equivalent resulted in the highest increase in pod yield, which was significantly different from that of 12.5, 25.0 and 50.0 mg/litre BA equivalent (Table 2). Accel at 12.5 mg/litre did not significantly increase pod yield compared to the control (Table 2). Further analysis revealed that the response to increasing Accel concentration was cubic (Table 2). In experiment 2, application of Accel at 12.5, 25.0, 37.5 or 50.0 mg/litre BA equivalent all significantly increased pod yield (Table 2). There were no significant differences between the effects of Accel at 25.0and 50.0 mg/litre BA equivalent on pod yield (Table 2). The response to increasing Accel concentration was linear. In both experiments 1 and 2, application of 37.5 mg/litre BA equivalent resulted in the highest yield; at higher concentration of Accel, there was a reduction in yield (Table 2).

4.3 Effect of BA on Pod Length, Pod Number and Fresh Pod Yield

In both experiments 1 and 2, benzyladenine (BA) had no significant effect on pod length and pod number (Table 3). In experiment 2, BA at 37.5 mg/litre tended to increase pod yield compared to the control; however, the effect was not statistically significant. Benzyadenine had no significant effect on fresh pod yield in experiment 1.

4.4 Effect of GA_{4+7} on Chlorophyll Content and Retention

4.4.1 Leaf and pod chlorophyll content

In experiment 1, GA_{4+7} had no significant effect on leaf and pod chorophyll content. In experiment 2, GA_{4+7} at 2.5, 3.75 and 5.00 mg/litre tended to enhance leaf and pod chlorophyll content, but this was not statistically significant (Table 4).

Table 3. Effect of BA on pod length, Pod Number per Plant and Pod Yield of 'Monel' French Beans.

FIRST EXEPERIMENT				SECOND EXEPERIMENT		
BA mg/l	**Pod length (cm)**	**Pod number**	**Yield (tons/ha)**	**Pod length (cm)**	**Pod number**	**Yield (tons/ha)**
0.00	0.00a	0.00a	0.00a	0.00a	0.00a	0.00b
12.50	-0.18a	2.50a	0.97a	0.15a	1.63a	0.12b
25.00	0.12a	0.00a	0.79a	0.17a	0.00a	0.98ba
37.50	-0.30a	1.28a	1.08a	0.14a	0.00a	2.16a
50.00	0.00a	2.30a	0.20a	0.00a	0.40a	1.41ba
Significance	ns	ns	ns	ns	ns	ns
LSD	0.49	3.41	1.70	0.54	2.21	1.86

ns significance within is non significant

Means were separated by the protected LSD (P=0.05); Means with the same letter(s) within columns are not significantly different.

4.4.2 Chlorophyll retention

In experiment 1, GA_{4+7} at 5.00 mg/litre significantly enhanced chlorophyll retention by the pods after storage (Table 4). GA_{4+7} at 5.00 mg/litre also tended to enhance chlorophyll retention by the pods in experiment 2 but this was not statistically significant. Application of GA_{4+7} at 1.25, 2.50 and 3.75 mg/litre had no significant effect on chlorophyll retention by the pods after storage (Table 4).

4.5 Effect of Accel on Chlorophyll Content

4.5.1 Leaf chlorophyll content

In both experiments 1 and 2, Accel application at 37.5 and 50.0 mg/litre BA equivalent significantly increased leaf chlorophyll content (Table 5). Accel at 12.5 and 25.0 mg/litre BA equivalent had no significant effect on leaf chlorophyll content; however, 25.0 mg/litre BA equivalent was not significantly different from either 37.5 or 50.0 mg/litre BA equivalent with respect to increasing leaf chlorophyll content (Table 5).

4.5.2 Pod chlorophyll content

In both experiments 1 and 2, application of Accel significantly increased pod chlorophyll content. In experiment 1, the response to increasing Accel concentration was linear (Table 5). In experiment 2, Accel at 12.5, 25.0 or 37.5 mg/litre BA equivalent significantly increased pod chlorophyll content compared to the control, but their effects were not significantly different from each other (Table 5). Application of Accel at 50.0 mg/litre BA

Table 4. Effect of GA_{4+7} on French Bean Pod and leaf Chlorophyll Content and Retention

FIRST EXEPERIMENT				SECOND EXPERIMENT		
GA_{4+7} mg/l	**Leaf chl.**	**Pod chl.**	**Pod Chl. after storage**	**Leaf chl.**	**Pod chl.**	**Pod Chl. after storage**
0	37.57a	11.94a	5.97b	38.81a	9.95b	5.22b
1.25	38.81a	11.94a	7.22ba	36.82a	9.95b	5.97ba
2.50	41.05a	12.19a	6.97b	42.05a	12.44a	6.72ba
3.75	41.05a	13.68a	6.97b	40.55a	11.20ba	6.97ba
5.0	42.05a	13.19a	8.71a	39.81a	12.19ba	7.46a
Significance	ns	ns	*	ns	ns	ns
LSD	5.22	2.24	1.74	3.68	2.24	1.74

*, ns, significance within columns at P=0.05 or not significant respectively.

Means were separated by the Protected Least Significance Difference (LSD) at P= 0.05; means followed by the same letter(s) within columns are not significantly different.

Table 5. Effect of Accel on French Bean Pod and leaf Chlorophyll Content and Retention

FIRST EXEPERIMENT				SECOND EXPERIMENT		
Accel mg/l	**Leaf chl.**	**Pod chl.**	**Pod Chl. after storage**	**Leaf chl.**	**Pod chl.**	**Pod Chl. after storage**
0	37.57b	11.94c	6.47c	38.81b	9.95c	5.22c
12.5	41.05ba	14.43b	8.96b	38.07b	12.94b	7.71b
25.0	41.05ba	15.67ba	9.70b	40.06ba	14.68ba	8.71b
37.5	44.29a	15.67ba	10.70ba	42.54a	15.43ba	9.70ba
50.0	44.54a	17.66a	12.19a	42.54a	16.67a	11.69a
Significance[z]	**	L**	L***	*	***	L***
LSD	3.73	2.49	1.74	3.23	2.74	1.99

[z] the response was linear (L)

*, **, ***, significance within columns at, 0.05,0.01 and 0.001 respectively.

Means separated by the Protected Least Significance Difference (LSD) at P= 0.05; means followed by the same letter(s) within columns are not significantly different.

4.5.3 Chlorophyll retention

Application of Accel significantly increased chlorophyll retention by French bean pods after 8 days of storage (Table 5). In both experiments 1 and 2, Accel at 50.0 mg/litre BA equivalent resulted in the highest pod chlorophyll retention. Accel at 12.5, 25.0 or 37.5 mg/litre BA equivalent also increased chlorophyll retention compared to the control but their effects were not significantly different from each other (Table 5). The response to increasing Accel concentration was linear in both experiments (Table 5).

4.6 Effect of GA_{4+7} on the Water Content of French Bean Pods

In both experiments 1 and 2, GA_{4+7} application significantly increased water content of pods (Table 6). In experiment 1, GA_{4+7} at 3.75 and 5.0 mg/litre resulted in the highest water content of pods, but there were no significant differences among them (Table 6). GA_{4+7} at 1.25 and 2.50 mg/litre also increased the water content of pods compared to the control, but their effects were not significantly different from each other (Table 6). In experiment 2, application of GA_{4+7} at 1.25, 2.5, 3.75 or 5.0 mg/litre increased the water content of French bean pods compared to the control but their effects were not significantly different from each other. The response to increasing GA_{4+7} concentration in both experiments 1 and 2 was linear (Table 6).

4.7 Effect of Accel on the Water Content of French Bean Pods

In both experiments 1 and 2, application of Accel at 25.0, 37.5 or 50.0 mg/litre BA equivalent significantly increased the water content of pods (Table 7). In both experiments Accel at 12.5 mg/litre BA equivalent did not significantly increase the pod water content compared to the control (Table 7).

Table 6. Effect of GA_{4+7} on Water Content (percent) of French Bean Pods

FIRST EXPERIMENT		SECOND EXPERIMENT
GA_{4+7} mg/l	Water content (%)	Water content (%)
0	86.56c	86.63b
1.25	87.88b	88.88a
2.5	88.75b	88.63a
3.75	90.31a	89.25a
5.0	90.5a	88.63a
Significance[z]	L***	L***
LSD	1.27	1.21

[z] the response was linear (L)

***, significance within columns at P= 0.001.

Means were separated by the Protected Least Significance Difference (LSD) at P= 0.05; means followed by the same letter(s) within columns are not significantly different.

Table 7. Effect of Accel on Water Content (percent) of French Bean Pods

First experiment		Second experiment
Accel mg/l	**Water content (%)**	**Water content (%)**
0	86.56b	86.63b
12.5	87.44b	87.31b
25.0	89.06a	89.63a
37.5	89.00a	89.06a
50.0	89.63a	88.94a
Significance	****	****
LSD	1.07	0.99

****, significance within columns at P= 0.0001

Means were separated by the Protected Least Significance Difference (LSD) at P= 0.05; means followed by the same letter(s) within columns are not significantly different.

Table 8. Effect of BA on Water Content (percent) of French Bean Pods

FIRST EXPERIMENT		SECOND EXPERIMENT
BA mg/l	**Water content (%)**	**Water content (%)**
0.0	0.00ba	0.00a
12.5	-0.44a	-1.56b
25.0	0.31a	1.00a
37.5	-1.31b	-0.19ba
50.0	-0.88ba	0.31a
Significance	ns	ns
LSD	1.34	1.40

ns, significance within columns = non significant

Means were separated by the Protected Least Significance Difference (LSD) at P= 0.05; means followed by the same letter(s) within columns are not significantly different.

4.8 Effect of BA on Water Content of French Beans

In both experiments 1 and 2 BA tended to depress water content of the pods; however, the BA effect was not statistically significant in both cases (Table 8).

4.9 Effect of GA_{4+7} on Wilting (%) of French Bean Pods

In experiment 1, application of GA_{4+7} did not significantly reduce the number of pods that wilted after 7 days of storage (Table 9). In experiment 2, GA_{4+7} application significantly reduced the number of pods that wilted after storage and the response to increasing concentration of GA_{4+7} was linear (Table 9).

4.10 Effect of Accel on Wilting (%) of French Bean Pods

In experiment 1, Accel at 25.0, 37.5 and 50.0 mg/litre BA equivalent significantly reduced the number of pods that wilted compared to the control. The effects of Accel at 37.5 and 50.0 mg/litre on pod wilting were not significantly different from each other (Table 10). Accel at 12.5 mg/litre did not significantly reduce wilting of pods compared to the control. The response to increasing Accel concentration was linear in experiment 1 (Table 10). In experiment 2, Accel at 37.5 mg/litre BA equivalent tended to reduce wilting of the pods, but the effect was not statistically significant (Table 10).

4.11 Effect of BA on Wilting (%) of French Bean Pods

In experiment 1, BA did not significantly affect wilting of pods (Table 11). In experiment 2, BA at 25.0 mg/litre significantly increased the number of pods that wilted (Table 11). However, BA at 12.5, 37.5 and 50.0 mg/litre did not significantly affect wilting of French bean pods in experiment 2 (Table 11).

Table 9. Effect of GA_{4+7} on the Percentage Wilting of French Bean Pods.

FIRST EXPERIMENT		SECOND EXPERIMENT
GA_{4+7} mg/l	Percentage of wilted pods	Percentage of wilted pods
0	28.19a	25.25a
1.25	26.88a	23.50b
2.5	26.50a	20.81d
3.75	26.38a	22.19c
5.0	26.44a	21.94dc
Significance [z]	ns	L****
LSD	2.08	1.3

[z] the response was linear (L)

****, ns, significance within columns at P= 0.0001 or not significant respectively

Means were separated by the Protected Least Significance Difference (LSD) at P= 0.05; means followed by the same letter(s) within columns are not significantly different.

Table 10. Effect of Accel on the Percentage Wilting of French Bean Pods.

FIRST EXPERIMENT		SECOND EXPERIMENT
ACCEL mg/l	**Percentage of wilted pods**	**Percentage of wilted pods**
0	28.19a	24.38a
12.5	26.69ba	24.38a
25.0	26.38b	23.31ba
37.5	24.69c	22.31b
5.0	24.19c	22.69ba
Significance[z]	L****	ns
LSD	1.53	1.76

[z] the response was linear (L)

****, ns, significance within columns at P= 0.0001 or non significant respectively

Means were separated by the Protected Least Significance Difference (LSD) at P= 0.05; means followed by the same letter(s) within columns are not significantly different.

Table 11. Effect of BA on the Percentage Wilting of French Bean Pods.

FIRST EXPERIMENT		SECOND EXPERIMENT
BA mg/l	WILTING	WILTING
0	0.00a	0.00b
12.5	-0.19a	0.88ba
25.0	-0.13a	2.50a
37.5	-2.06a	0.13b
50.0	-2.25a	0.75b
Significance	ns	*
LSD	2.33	1.75

*, ns, significance within columns at P= 0.05 or non significant respectively

Means were separated by the Protected Least Significance Difference (LSD) at P= 0.05; means followed by the same letter(s) within columns are not significantly different.

4.12 Effect of GA_{4+7} on Water Loss by French Bean Pods (Experiment 1)

Application of GA_{4+7} significantly reduced the rate of water loss by the French bean pods (Figure 1). After 1 day of storage, GA_{4+7} at 2.50, 3.75 and 5.0 mg/litre reduced water loss in the pods of French beans but their effects were not significantly different from each other. GA_{4+7} at 1.25 mg/litre did not significantly reduce the rate of water loss compared to the control. After 2 days of storage, GA_{4+7} at 1.25, 5.0, 3.75 or 5.0 mg/litre all significantly reduced the rate of water loss compared to the control (Figure 1). After 3 days of storage, GA_{4+7} application significantly reduced the rate of water loss, but the effect was not dependent on the concentration of GA_{4+7}; thus, the effects of all the 4 levels of GA_{4+7} were not significantly different from each other (Figure 1). After 4 days of storage, GA_{4+7} at 1.25, 2.50, 3.75 or 5.00 mg/litre all reduced the rate of water loss by the pods compared to the control (Figure 1). The effects of GA_{4+7} at 1.25 and 2.5 mg/litre were not significantly different from each other. GA_{4+7} at 3.75 and 5.0 mg/litre had the greatest effect on reduction of water loss, but their effects were not significantly different from each other (Figure 1). After 5 days of storage, GA_{4+7} at 1.25 and 2.50 mg/litre did not significantly reduce the rate of water loss by the pods. However, 3.75 and 5.0 mg/litre GA_{4+7} reduced water loss but their effects were not significaaantly different from each other (Figure 1). After 6 days of storage, GA_{4+7} at 1.25, 2.50, 3.75 or 5.0 mg/litre all reduced the rate of water loss of French bean pods (Figure 1). GA_{4+7} at 3.75 mg/litre had the greatest effect on reduction of the rate of water loss. After 7 days of storage, GA_{4+7} at 1.25, 2.50, 3.75 or 5.0 mg/litre significantly reduced the rate of water loss by the pods (Figure 1). The rate of water loss decreased with the increase in GA_{4+7} concentration (Figure 1).

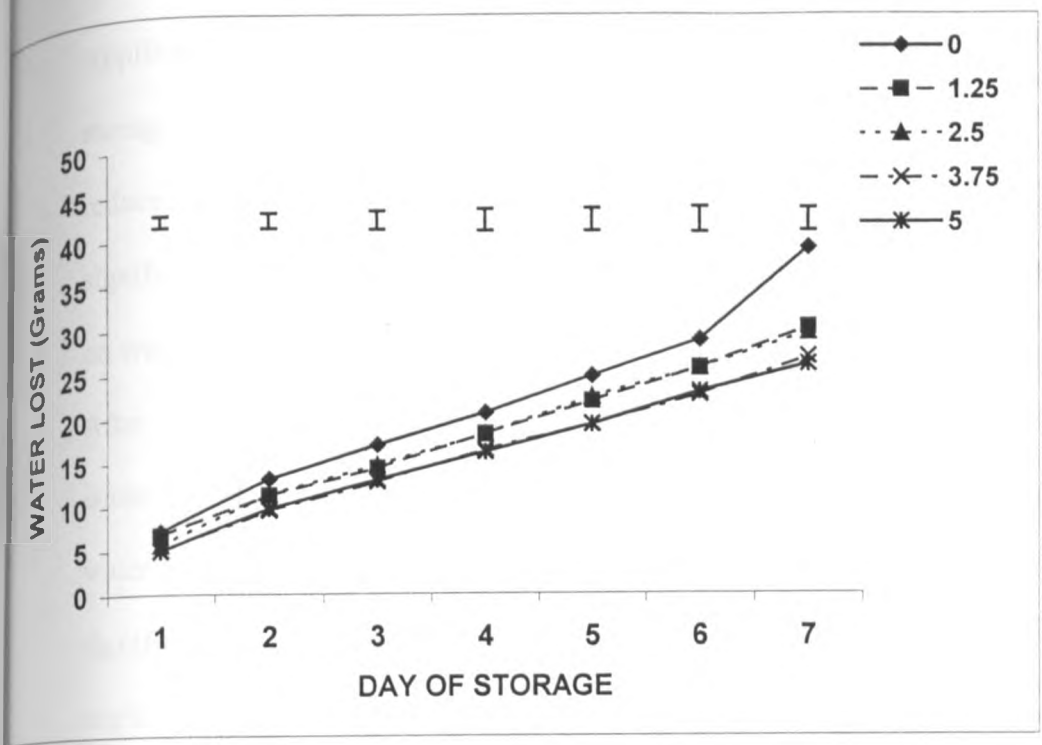

Figure 1. Effect of GA4+7 on the rate of water loss by French bean pods (Experiment 1). Vertical bars are LSD bars at P = 0.05

4.13 Effect of GA_{4+7} on Water Loss by French Bean Pods (Experiment 2)

Application of GA_{4+7} significantly reduced the rate of water loss at all the days of storage (Figure 2). After 1 day of storage, GA_{4+7} at 2.5, 3.75 or 5.0 mg/litre significantly reduced the rate of water loss by the pods, but their effects on water loss were not significantly different from each other. GA_{4+7} at 1.25 mg/litre had no significant effect on water loss (Figure 2).

After 2 days of storage, GA_{4+7} at 1.25, 2.5, 3.75 or 5.0 mg/litre all reduced the rate of water loss of French bean pods (Figure 2). GA_{4+7} at 2.50, 3.75 or 5.0 mg/litre reduced water loss in a similar trend (Figure 2). After 3 days of storage, all the GA_{4+7} levels significantly reduced the rate of water loss (Figure 2). GA_{4+7} at 2.50, 3.75 or 5.0 mg/litre reduced water loss of French beans and their effect were significantly different from each other (Figure 2). After 4, 5, 6 and 7 days of storage, the effect of GA_{4+7} showed a similar trend. GA_{4+7} at 2.5, 3.75 and 5.0 mg/litre all significantly reduced the rate of water loss (Figure 2). GA_{4+7} at 1.25 mg/litre had no significant effect on water loss by French bean pods after 4, 5, 6 and 7 days of storage (Figure 2).

4.14 Effect of Accel on water loss by French Bean Pods (Experiment 1)

In experiment 1, Accel significantly reduced the rate of water loss compared to the control (Figure 3). The trend for the effect of Accel on reduction of water loss after 1, 2, 3, and 4 days of air storage was similar (Figure 3). After the first 4 days of air storage, Accel at 25.0, 37.5 and 50.0 mg/litre significantly reduced the rate of water loss by the

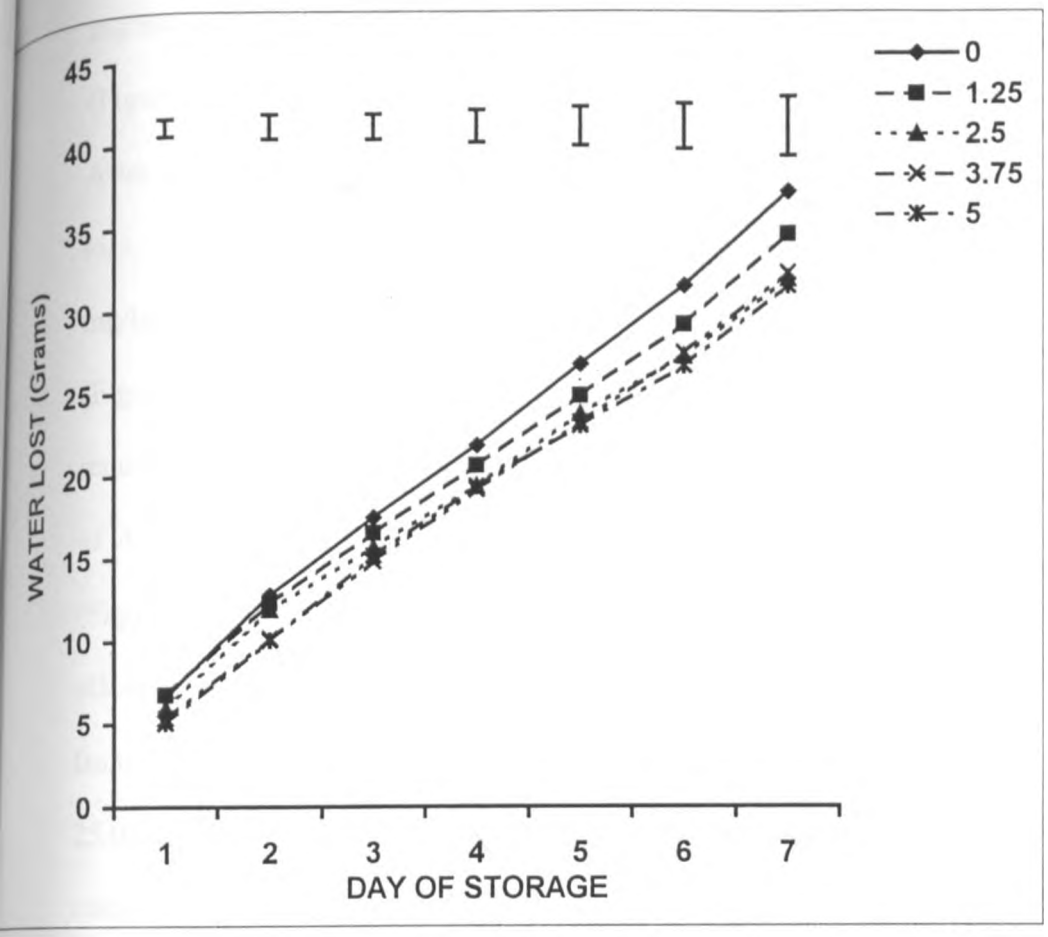

Figure 2. Effect of GA4+7 on the rate of water loss by French bean pods (Experiment 2). Vertical bars are LSD bars at P = 0.05

pods but their effects were not significantly different from each other. Accel at 12.5 mg/litre did not significantly reduce the rate of water loss compared to the control (Figure 3).

After 5 days of storage, Accel at 12.5, 25 or 37.5 mg/litre all reduced the rate of water loss, but their effects were not significantly different from each other. Accel at 50.0 mg/litre had the greatest effect on reduction of water loss by the French bean pods (Figure 3). After 6 days of storage, Accel at 25.0, 37.5 or 50.0 mg/litre all significantly reduced the rate of water loss of French bean pods compared to the control. The effects of Accel at 37.5 and 50.0 mg/litre BA equivalent were not different from each other (Figure 3). Accel at 12.5 mg/litre BA equivalent had no effect on water loss and its effect on reducing water loss of the pods of French beans was not significantly different from that of 25.0 mg/litre BA equivalent (Figure 3). After 7 days of storage, Accel at 25.0, 37.5 and 50.0 mg/litre BA equivalent significantly reduced the rate of water loss compared to the control (Figure 3). Accel at 12.5 mg/litre BA equivalent did not significantly reduce the rate of water loss (Figure 3)

4.15 Effect of Accel on Water Loss by French Bean Pods (Experiment 2)

Accel application had varied results on the rate of water loss by the pods of French beans during the 7 days of storage (Figure 4). After 1 day of storage, only level 37.5 mg/litre BA equivalent significantly reduced the rate of water loss compared to the control (Figure 4). After 2 days of storage, Accel at 37.5 and 50.0 mg/litre BA equivalent significantly reduced the rate of water loss by pods, but their effects were not significantly different from each other. After 3 days of storage, Accel at 12.5 mg/litre BA equivalent did not significantly reduce the rate of water loss compared to the control. Accel at 25.0, 37.5 and 50.0 mg/litre BA equivalent significantly reduced the

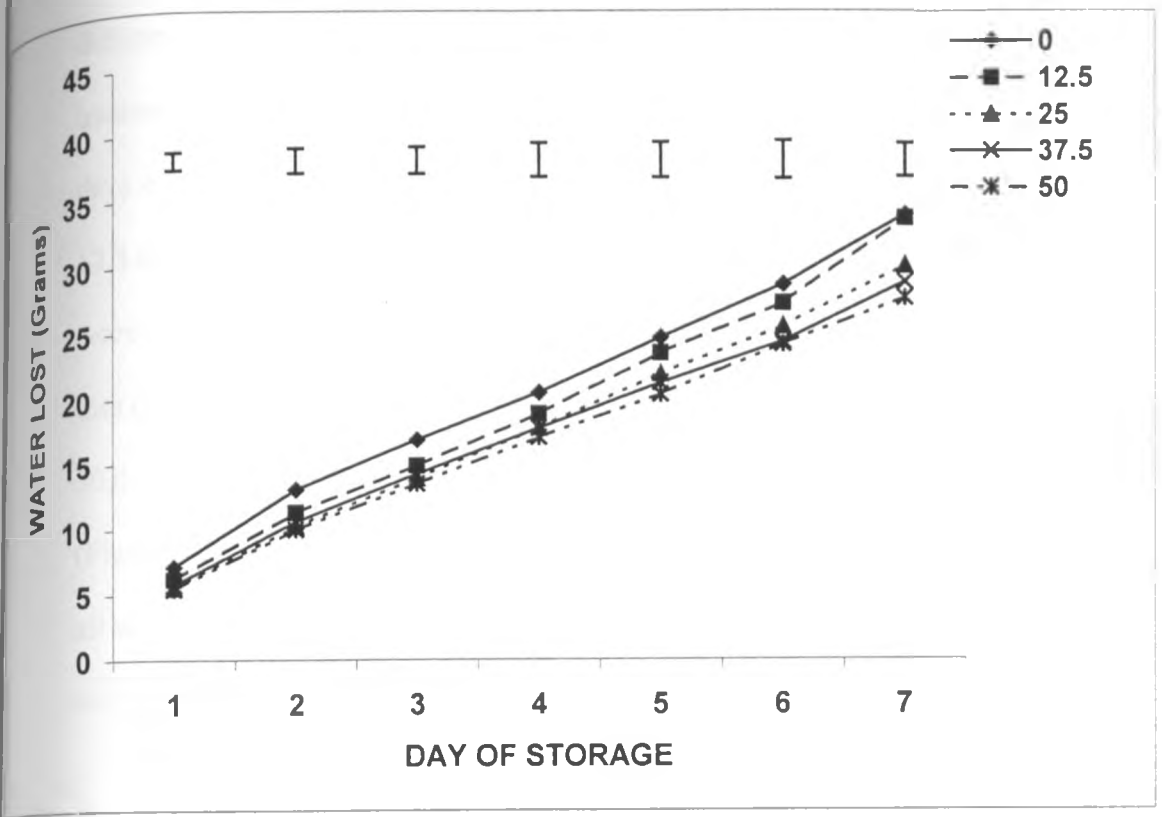

Figure 3. Effect of Accel on the rate of water loss by French bean pods (Experiment 1). Vertical bars are LSD bars at P = 0.05

rate of water loss in pods of French beans, but their effects were not significantly different from each other (Figure 4). Accel at 37.5 mg/litre BA equivalent was the only treatment that reduced the rate of water loss from pods of French beans after 4 and 5 days of air storage at room temperature (Figure 4). After 6 days of storage, Accel at 12.5 and 25.0 mg/litre BA equivalent did not significantly reduce rate of water loss. Accel at 37.5 and 50.0 mg/litre BA equivalent significantly reduced the rate of water loss (Figure 4). There was no significant concentration effect among levels 25.0, 37.5 or 50.0 mg/litre BA equivalent in response to pod water loss 6 days after air storage (Figure 4). After 7 days of storage, Accel at 25.0, 37.5 and 50.0 mg/litre BA equivalent all significantly reduced the rate of water loss. Accel at 12.5 mg/litre BA equivalent did not significantly reduce the rate of water loss by the pods of French beans (Figure 4).

4.16 Effect of BA on Water Loss by French Bean Pods (Experiment 1)

In experiment 1, BA had no significant effect on water loss of the pods by French beans, between 1 and 6 days of air storage (Figure 5). However after 7 days of storage, 12.5 mg/litre BA significantly enhanced water loss from the pods of French beans (Figure 5). The effect of 12.5 mg/litre BA was not significantly different from that of 37.5 and 50.0 mg/litre (Figure 5).

4.17 Effect of BA on Water Loss by French Bean Pods (Experiment 2)

In experiment 2, BA had no effect on water loss of French bean pods in all the 7 days of storage at room temperature (Figure 6).

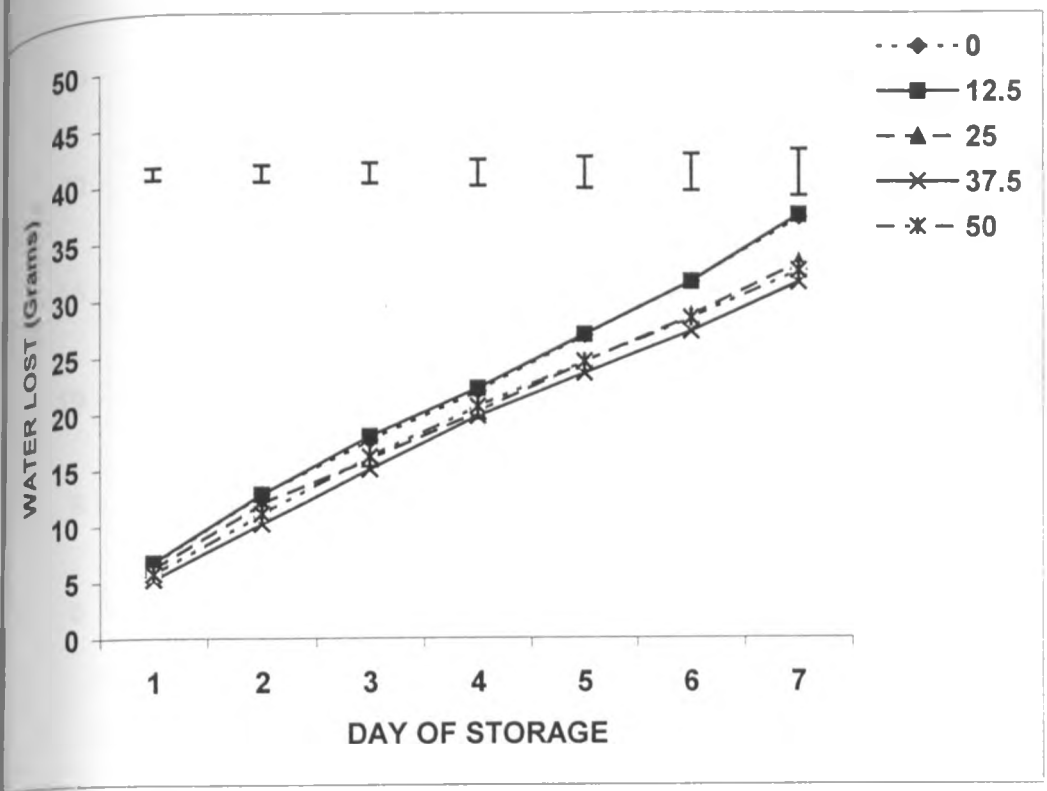

Figure 4. Effect of Accel on the rate of water loss by French bean pods (Experiment 2). Vertical bars are LSD bars at P = 0.05

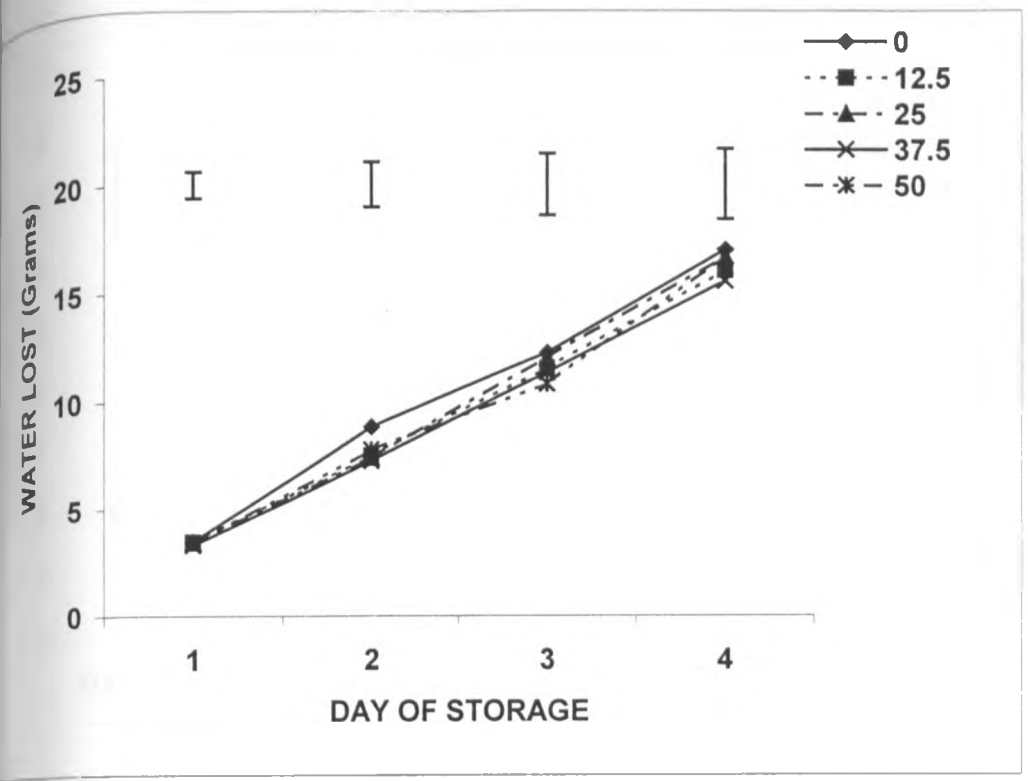

Figure 8. Effect of Accel on the rate of water loss by French beans

after cold storage at 4^0C. Vertical bars are LSD bars at P = 0.05

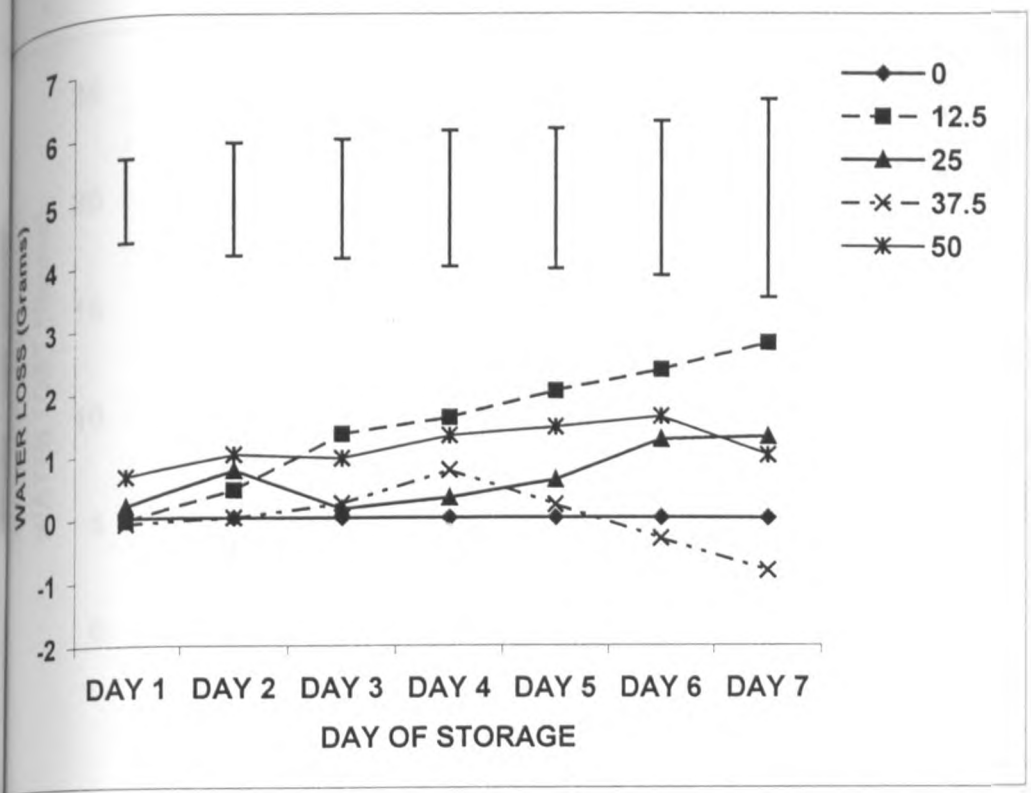

Figure 6. Effect of BA on the rate of water loss by French bean pods (Experiment 2). Vertical bars are LSD bars at P = 0.05

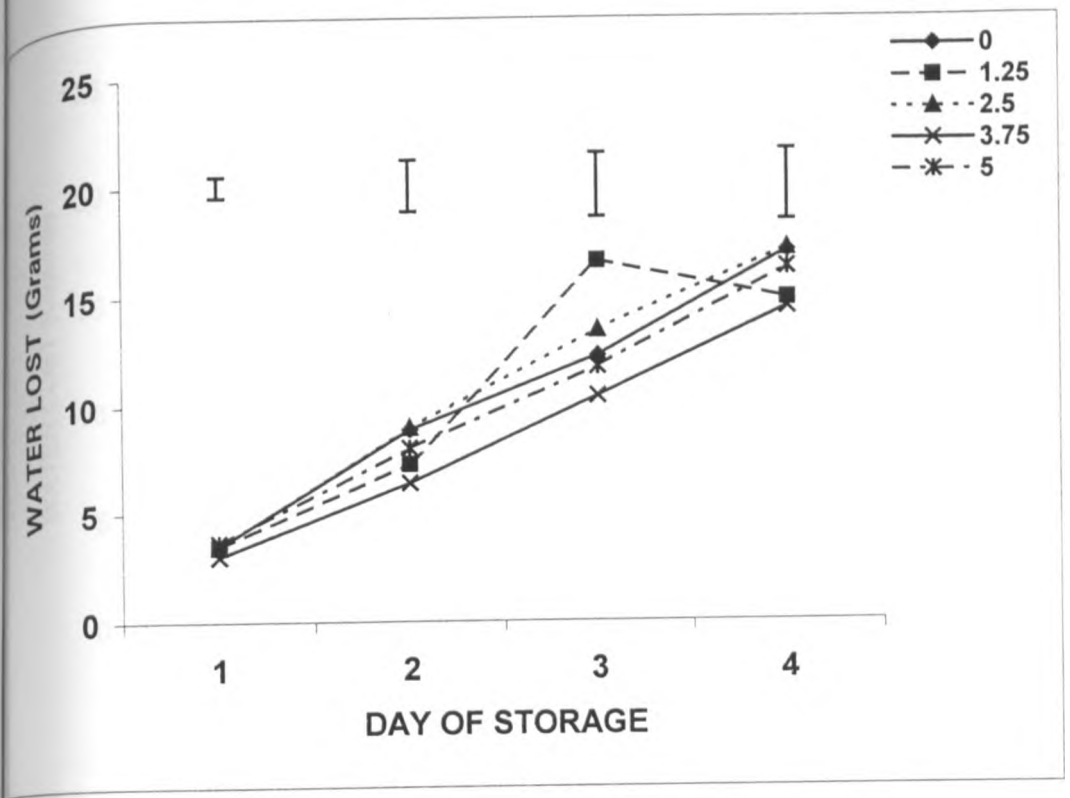

Figure 7. Effect of GA4+7 on the rate of water loss by French beans after cold storage at 4^0C. Vertical bars are LSD bars at P = 0.05

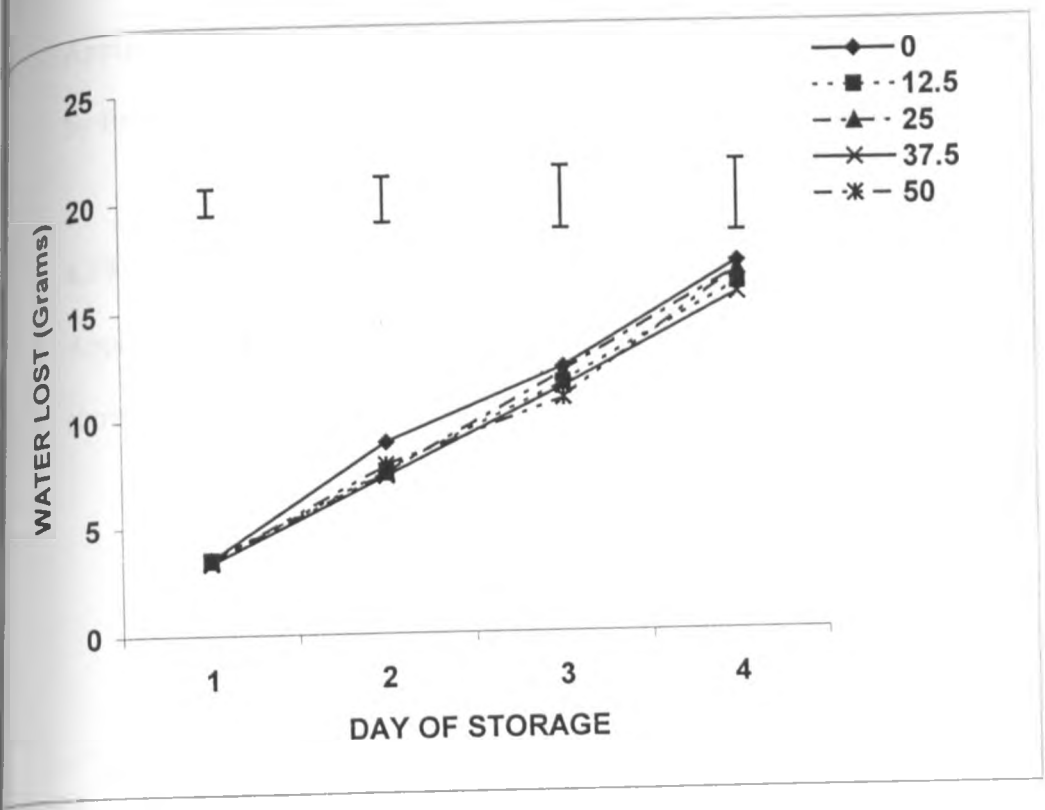

Figure 8. Effect of Accel on the rate of water loss by French beans after cold storage at 4^0C. Vertical bars are LSD bars at P = 0.05

4.18 Effect of GA_{4+7} and Accel on Post Storage Water Loss by Pods

Application of both GA_{4+7} and Accel had no significant effect on the rate of water loss by French bean pods after 21 days of cold storage at 4^0C (Figure 7 and 8).

4.19 Effect of GA_{4+7} and Accel on Chilling Injury

Application of GA_{4+7} and Accel had no significant effect on the number of pods that showed chilling injury symptoms, 21 days after cold storage at 4^0C (Table12 and 13).

Table 12. Effect of GA_{4+7} on the Percentage Chilling Injury of French Bean Pods

GA_{4+7} mg/l	Percentage Chilling
0	22.69a
1.25	22.19a
2.5	22.69a
3.75	22.50a
4.0	22.88a
Significance	ns
LSD	2.13

ns, significance within columns is not significant

Means were separated by the Protected Least Significance Difference (LSD) at P= 0.05;

Means followed by the same letter(s) within columns are not significantly different.

Table 13. Effect of Accel on the Percentage Chilling Injury of French bean pods

ACCEL mg/l	**Percentage Chilling**
0	22.69a
12.5	22.38a
25.0	22.00a
37.5	22.38a
5.0	22.75a
Significance	ns
LSD	2.19

ns, significance within columns is not significant

Means were separated by the Protected Least Significance Difference (LSD) at P= 0.05;

Means followed by the same letter(s) within columns are not significantly different.

CHAPTER FIVE

DISCUSSION

5.1 Effect of GA, Accel and BA on Pod Length.

In both experiments 1 and 2, GA_{4+7} at 1.25, 2,5, 3.75 or 5.0 mg/litre significantly increased pod length. This effect can be attributed to the effect of gibberellins on cell and tissue elongation (Baninasab and Rahemi, 1998; Potter *et al.*, 1999; Bagatharia and Chanda, 1998). The effect of GAs on tissue elongation is a result of enhanced cell elongation and partially cell division (Salisbury and Ross, 1990). In this study application of GA_{4+7} independently (as Provide) or together with BA (as Accel) may have enhanced elongation of the French bean cells, thereby leading to the increased pod length observed.

Gibberellins promote cell division by stimulating cells in G_1 phase to enter the S phase and by shortening the S phase, resulting in faster cell division (Liu and Loy, 1976). Gibberellins also increase hydrolysis of starch, fructans and sucrose into glucose and fructose molecules. These hexoses provide energy via respiration and also contribute to cell wall formation, thereby promoting cell growth (Salisbury and Ross, 1990). The hexoses also make the cell's water potential momentarily more negative, hence rapid entry of water, causing cell expansion, but diluting the sugars (Salisbury and Ross, 1990). Gibberellins also cause increased synthesis of invertase enzymes that hydrolyze incoming sucrose into glucose and fructose in some species such as sugarcane, thereby making the cell's water potential more negative, which in turn results in increased water uptake by the cells (Glasziou, 1969). In the present study, application of GA_{4+7} resulted in increased water uptake by the cells of French beans. As a result, the treated pods had

higher water content. The enhanced water uptake by the cells possibly led to enhanced cell expansion, hence the increased pod length observed in this study.

The enhanced pod length observed could also be attributed to the GA effect on cell wall plasticity whose net result is elongation growth (Salisbury and Ross, 1990). Studies in lettuce hypocotyls have indicated that the ultimate action of GA in this tissue is to bring about increased wall plasticity or to maintain the cell walls in a plastic state for a longer time (Stuart and Jones, 1977).

Recent studies have shown that hypocotyls fed with tritiated GA_1 accumulate significant amounts of labile in a purified cell wall fraction and that this process precedes growth response (Stoddart, 1981). This taken with the extensive physiological data showing changes in wall properties suggests that the action of GA may be related to the postponement of the formation of non-labile wall linkages, which ultimately preclude further extension growth. This is supported by the fact that ageing of hypocotyls progressively diminishes the response to a given dose of gibberellins (Stoddart, 1981).

Accel application increased pod length in the present study. However, separation of the BA effect from that of GA_{4+7} to determine the independent effect of BA on pod length showed that BA alone did not enhance pod length. This shows that the effect of Accel on pod length is a gibberellin effect, but not a cytokinin effect.

French beans are valued for their long pods; pods which are shorter than 10 cm are considered unfit, especially for the export market. Pod length is important in French

beans targeting the export market, because there are standard containers that are used to package the French beans for export. The tips of the pods are normally trimmed before packaging. This means that when the pods are shorter than the container, they are considered to be rejects. The longer pods are preferred, because they can be trimmed to fit the packaging container.

5.2 Effect of GA_{4+7}, Accel and BA on Pod Number

The results of this study showed that GA_{4+7} increased the number of pods per plant. Accel also increased pod number and the response was cubic in experiment 1 and linear in experiment 2. BA alone did not have a significant effect on pod number. This suggests that the effect of Accel on pod number was a GA_{4+7} effect and not a BA effect. However, BA and GA_{4+7} tended to have a synergistic effect on pod number, with application of Accel resulting in a higher pod number compared to that of GA_{4+7} alone.

Gibberellins (GA_{4+7}) and BA have been shown to increase pod set and subsequently pod number per branch in many leguminous crops (Bruce, 1990; Nowak *et al.*, 1997; Dybing and West Gate, 1996). Cytokinin treatment has been reported to enhance pod set in French bean varieties that fail to set pods in the summer (Morgan, 1981). Gibberellins have the ability to increase the fruit bearing nodes which eventually develop into pods (Belluci *et al.*, 1982, Abdel-Fattah *et al.*, 1995). Gibberellins have also been reported to reduce the time to flowering and increase the number of flowers per plant (Shahine *et al.*, 1992). An increase in the number of flowers per plant is a positive indicator of high pod yield. Gibberellins have also been reported to reduce abscission of flowers and pods in water-stressed beans (Abdel-Fattah *et al.*, 1995), thereby increasing number of pods that reach maturity. Application of cytokinins has

also been reported to prevent flower bud abscission in French beans during long days (Morgan, 1981). In the present study, the increased pod number per plant observed following GA_{4+7} and BA application can be attributed to their ability to increase the number of flowers hence, high pod set and the number of pods that reached maturity. The increase in pod number can also be attributed to enhanced sink strength resulting in increased assimilate accretion following GA_{4+7} and BA treatment. Application of BA and GA_{4+7} also increased leaf chlorophyll content. This increase in leaf chlorophyll per unit leaf area increased pod number possibly by increasing the rate of photosythesis.

5.3 Effect of GA_{4+7}, Accel and BA on Pod Yield

In both experiments 1 and 2, GA_{4+7} at 1.25, 2.50, 3.75 or 5.0 mg/litre significantly increased the pod yield of French beans. In both experiments 1 and 2, Accel at 25.0, 37.5 and 50.0 mg/litre BA equivalent significantly increased the yield of French bean pods. BA alone had no significant effect on pod yield; however, GA_{4+7} + BA tended to have a synergistic effect on pod yield. In this study, GA_{4+7} independently and synergistically with BA in Accel increased pod length and number of French beans. The increase in pod length and number contributed to the increased pod yield observed, because pod length and number are yield components of French beans. Accel also increased pod yield of French beans probably because of the increase in chlorophyll content per unit leaf area. This possibly resulted in an increase in the rate of photosynthesis and accumulation of dry matter, hence pod yield.

Gibberellins and BA have been reported to increase leaf number, leaf area index (LAI) and leaf area duration of many legumes (Harb, 1992). A high leaf area index exposes a

larger area for CO_2 fixation, resulting in enhanced plant growth and development rate, hence enhanced dry matter accumulation (Gardner *et al.*, 1985). Enhanced dry matter accumulation by the French bean plants following application of BA (Accel) and GA_{4+7} may have resulted in the increased pod yield observed.

Dry matter distribution significantly contributes to the yield potential of crop plants and this largely accounts for the increased yields of cultivars over their ancestral stocks. Phytohormones have been reported to enhance dry matter accretion by the sink in several species such as peach fruit (Patrick and Wareing, 1981). Application of GA_{4+7} and Accel may have, therefore influenced dry matter distribution in the French bean plants in favour of the pods, leading to the increased pod yield observed.

The rate of supply and distribution of carbon assimilates to and within developing flowers and pods determine the numbers and sizes of the various yield components (Morgan, 1981). In the present study, application of BA in Accel may have increased loading and unloading of assimilates across membrane boundaries of the vascular tissues of French bean plants, leading to increased dry matter production (Clifford *et al.*, 1986). The increased dry matter production may have resulted in the increased yield observed in the French beans. BA may have also promoted carbohydrate metabolism and created new source-sink relationships, leading to increased dry matter accumulation in the French bean pods (Mothes and Engelbretcht, 1961; Dyer *et al.*, 1990).

The increase in pod yield observed in this study as a result Accel and GA_{4+7} application can therefore be attributed to the effect of these PGRs on dry matter accumulation and on assimilate mobilization and /or distribution. The increase in pod yield can also be

attributed to the increased pod water content observed in the present study since fresh weights were used to estimate the pod yield of French beans.

5.4 Effect of GA_{4+7}, Accel and BA on Chlorophyll Content, Chlorophyll Retention and Senescence of French Beans.

Application of Accel significantly increased chlorophyll content and retention in leaves and pods of French beans. In both experiments, the increase in chlorophyll retention was linear with the higher concentration of Accel causing a higher content and retention of chlorophyll. Gibberellins had no significant effect on chlorophyll content of pods and leaves in both experiments. Given that GA_{4+7} had no significant effect on chlorophyll content of leaves and pods, the increased leaf and pod chlorophyll content/retention observed following Accel application was due to benzyladenine (a cytokinin).

Cytokinins delay senescence primarily through their effect on chlorophyll and protein synthesis and/or retention by plant tissues. Leaf yellowing, which is attributed to a decline in chlorophyll, provides an easy and convenient measure of the progress of leaf senescence. Chemical analyses show that leaf senescence is accompanied by early losses in chlorophyll, RNA and proteins, including many enzymes. These losses could be a result of slower synthesis and/or faster breakdown of the chlorophyll, RNA and proteins (Salisbury and Ross, 1990; Humbeck *et al.*, 1996). Yellowing of detached plant tissues such as leaves also results from the breakdown of chlorophyll, which exposes other plant pigments such as xanthophylls and carotenoids (Leopold *et al.*, 1959). In the present study, BA may have increased the chlorophyll content of French bean pods and leaves by slowing the loss of these macromolecules by the tissues of French beans.

Accel (BA) application delayed yellowing of the French bean pods through its effect on chlorophyll retention by treated tissues. In the present study, Accel (BA) application enhanced chlorophyll retention by French bean pods after storage.

Cytokinins have been shown to delay and/or inhibit chlorophyll breakdown by reducing the rate of protein degradation during senescence (Sacher, 1973; Van Staden and Joughin, 1988). Discolouration in cut-flowers as a result of chlorophyll breakdown and exposure of other plant pigments like xanthophylls and carotenoids can be reduced by foliar sprays of cytokinins (Halevy and Mayak, 1981; Healy and Lang, 1989). Cytokinins promote chloroplast development and chlorophyll synthesis (Momotani *et al*, 1991). Benzyladenine has been reported to delay the degradation of chlorophyll by retarding the breakdown of proteins used in chlorophyll synthesis (Mutui, 1999; Van Staden and Joughin, 1988).

Cytokinins have the ability to enhance development of etioplasts into choloroplasts and to increase the rate of chlorophyll formation. They enhance formation of proteins onto which chlorophyll binds and becomes stabilized (Lew and Tsuji, 1982; Salisbury and Ross, 1990).

The primary step in the degradation of the soluble type ribonucleic acid (RNA) is thought to involve loss of the end adenine (Salunke *et al.*, 1962). It is believed that one of the mechanisms by which BA delays senescence is by providing the necessary adenine and restoring the soluble RNA molecule. This should in turn retard protein breakdown so that the treated produce stays fresh for a longer time (Mutui, 1999). This effect was evidenced by high nitrogen retention in the leaves of Alstromeria treated

with Accel (Mutui, 1999). The role of cytokinins in senescence could probably be through maintenance of high RNA and protein levels (Ballantyne, 1966)

Cytokinins also enhance chlorophyll content and retention by promoting grana formation (Lew and Tsuji, 1982). Grana are the stacks of thylakoids, which contain photosynthetic pigments namely chlorophyll a, chlorophyll b and carotenoids (Salisbury and Ross, 1985). An increase in the volume of grana consequently results in an increase in the rate of chlorophyll formation (Lew and Tsuji, 1982). Benzyladenine has also been shown to increase chloroplast DNA content (Momotani *et al.*, 1991). An increase in the volume of grana coupled with a high chloroplast DNA content may explain the enhanced chlorophyll retention by French bean pods observed following Accel application.

In citrus fruits, BA and GA_3 were shown to inhibit the degreening effect caused by ethylene (Dostal and Leopold, 1967). The degreening effect is a result of *de novo* synthesis of chlorophyllase protein and increase of chlorophyllase enzyme activity. Gibberellins (GA_3), at concentrations as low as 0.1 mg /litre, effectively delayed degreening of detached citrus fruits and that this effect lasted longer when GA_3 was sprayed on the tree (Goldschmidt 1973). Similar effects have been reported in Valencia oranges. This suggests that gibberellins not only act as inhibitors of senescence but also as inducers of green chloropast development (Goldschmidt, 1973).

5.5 Effect of GA_{4+7}, Accel and BA on Water Content of Pods

In both experiments 1 and 2, GA_{4+7} at 1.25, 2.50, 3.75 or 5.0 mg/litre all enhanced the water content of pods and the response was linear. Gibberellins have been reported to

promote fresh weight at the expense of dry weight of plant tissues (Salisbury and Ross, 1990). Eid and Ahmed (1976) and Sadowska *et al.*, (1983) reported increased succulence in sweet basil (*Ocimum basilicum* L.) following GA_3 treatment. Mutui (1999) reported that GA_{4+7} reduced the dry weight of Alstromeria leaves and increased the leaf and flower water content. Treatment of bean seeds with GA at 100 ppm and CEPA (ethephon) at 100 ppm were reported to result in increased water uptake, respiration rate, water retention capacity and catalase activity (Toa – HanZhi *et al.*, 1995). The treatments also resulted in a higher TSS, sucrose and soluble protein contents in the cotyledons compared with the controls. The enhanced content of sugars such as sucrose makes the cells' osmotic potential more negative, thereby increasing uptake of water by the cells. Gibberellins increase hydrolysis of the principal leaf storage products, accumulating in light namely starch, fructans and sucrose into glucose and fructose molecules Salisbury and Ross (1990). These hexoses in turn increase the cell's osmotic potential, making the cell to draw in water from its environment. This results in increased succulence of treated tissues (Salisbury and Ross, 1990). This could account for the enhanced water content of the French bean pods following GA_{4+7} application.

Accel at 25, 37.5 and 50.0 mg/litre BA equivalent significantly increased the water content of pods. Benzyladenine alone tended to have a depressing effect on the water content of pods. This implies that the enhanced water content observed following Accel application was a gibberellin (GA_{4+7}) but not a cytokinin (BA) effect. Cytokinins are known to enhance dry matter production (Salisbury and Ross, 1990; Emongor, 1995). Mutui (1999) reported that high levels of benzyladenine (75 or 100mg/litre) significantly decreased Alstromeria leaf water content, but increased leaf dry matter. In

this study, Benzyladenine (BA) depressed pod water content, because it promoted dry matter accumulation as evidenced by increased pod yield; water content and dry matter are negatively correlated (Emongor, 1997).

5.6 Effect of Accel, GA_{4+7}, and BA on Water Loss and Wilting

In experiment 2, GA_{4+7} reduced the number of pods that wilted. The response of wilting to GA_{4+7} was linear. In both experiments 1 and 2, GA_{4+7} significantly reduced the rate of water loss by the pods. Higher Accel concentrations (25.0, 37.5 or 50.0 mg/litre BA equivalent) significantly reduced the number of pods that wilted and also reduced the rate of water loss by the pods. This reduction in wilting could be as a result of the enhanced water content of the pods following GA_{4+7} and GA_{4+7}+BA treatment. The French bean plants treated with either GA_{4+7} or GA_{4+7}+BA resulted in pods with a higher water content and therefore they retained more water than pods from untreated plants.

One characteristic feature of senescing detached plant tissues such as leaves, pods and flowers is wilting as a result of water loss. Longevity of such tissues depends on their ability to maintain turgidity. Turgidity is determined by the balance between the rate of water loss (utilization) and of water supply (Mutui, 1999). Turgidity is necessary for the continuance of normal metabolic activities in the cells (Rogers, 1973). PGRs have been shown to influence water relations in detached plant tissues such as cut-flowers (Mayak and Halevy, 1974). In cut roses, kinetin maintained water balance by enhancing water uptake, therefore delaying wilting (Mayak and Halevy, 1974). The enhanced water uptake served to maintain cell integrity. In gerbera cut-flowers, BA delayed the

decrease in water content and increased ion leakage associated with senescence, thereby maintaining cell integrity (Van Meeteren, 1979).

CHAPTER SIX

CONCLUSIONS

The current world population explosion demands that alternative measures of increasing crop production be sought. With increased knowledge of plant physiology, crop improvement is tending towards new technological approaches such as the use of PGRs and genetic engineering. Plant growth regulators have been used commercially since the 1950s to modify plant growth, enhance yield, alter harvesting patterns and improve mechanical harvesting (Emongor, 1997).

In this study, both Accel and GA_{4+7} significantly increased yield and yield components (pod number and pod length). Although Accel and GA_{4+7} enhanced pod yield in this study, further research is required to determine the timing of application of both Accel and GA_{4+7} and the optimum concentration under different growing conditions of French beans.

Accel and GA_{4+7} application enhanced pod length which is welcome especially to dealers targeting the export market. Longer pods are preferred to shorter ones because there are standard packaging containers for respective export markets for French beans. Before packaging, pods are trimmed on both ends so as to fit in the packaging containers which are specific to the target market (HCDA, 1996). It is necessary that pods be long enough ($\geq$ 10 cm) so that after trimming it can still fit into the standard containers. For this reason the shorter pods are normally discarded or sold locally. The use of Accel and/or GA_{4+7} to increase pod length of French beans can help to reduce the export rejects.

In this study, Accel was shown to significantly improve the shelf-life of French bean pods as a result of its effect on chlorophyll retention. Soon after harvest, French bean pods begin to loss their chlorophyll and start to yellow thereby losing their aesthetic value. Fast deterioration of French bean pods after harvest is a major problem to both the exporters and retailers. Improvement of the shelf-life of the pods would greatly reduce the postharvest losses incurred as a result of yellowing. Studies in transgenic plants reveal that if cytokinin production is precisely controlled in a quantitative, spatial and temporal manner senescence can be delayed without other developmental alterations.

Both GA_{4+7} and Accel significantly reduced the rate of water loss by the pods and increased their water uptake/content. Water constitutes more than 70% of the fresh weight of most fresh horticultural produce such as vegetables, fruits and flowers. French beans, like other horticultural commodities have a high water content at harvest. They rapidly lose this water after harvest causing them to wilt and lose their aesthetic value and saleable weight. The use of GA_{4+7} and Accel to reduce postharvest water loss of French bean pods needs to be explored further to determine their feasibility for commercial use. Economic use of the PGRs like GA_{4+7} and Accel in this aspect will ensure that the saleable weight of fresh produce lost as water after harvest is retained. This will also contribute to reduction of postharvest losses as a result of wilting.

From the results of this study, it is concluded that both GA_{4+7} and Accel have the potential to be used in the culture of French beans in Kenya. The recommended concentrations are between 2.50 to 3.75 mg/l for GA_{4+7} and 25.0 to 37.5 mg/l for Accel. This is because the differences in the various variables following application of the

PGRs at these concentrations were not significantly different from the higher concentration, 5.0 mg/l and 50.0 mg/l for GA_{4+7} and Accel respectively. It would be necessary to do gross margin analyses to determine if the benefits from the application of these PGRs justify the cost. This is because the high cost of PGRs is one factor that has hampered their use in agricultural production. The effect of these PGRs on the nutritive value of the French beans needs to be determined before they can be adopted for use in agricultural production. The western countries, where most of the French beans are exported are very sensitive to traces of chemicals in foods and therefore it is necessary to determine if there are any residues of these PGRs in the French bean pods before they can be used in production.

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APPENDICES

A1. ANOVA table for the effect of GA_{4+7} on pod length of French beans

MEAN SUM OF SQUARES

Source of variation	df	Experiment 1	Experiment 2
Block	3	0.08 ns	0.39 **
Treatment	4	0.21 ***	0.24 **
Error	12	0.027	0.04
Corrected total	19	1.43	2.63
CV		1.48	1.85

df = degrees of freedom
CV = % coefficient of variation
, *, ns = significant at 0.01, 0.001 and not significant, respectively.

A2. ANOVA table for the effect of GA_{4+7} on pod number of French beans

MEAN SUM OF SQUARES

Source of variation	df	Experiment 1	Experiment 2
Block	3	21.00 ns	13.08 **
Treatment	4	79.96 ****	55.72 ****
Error	12	6.70	1.92
Corrected total	19	463.29	285.19
CV		4.19	2.27

df = degrees of freedom
CV = % coefficient of variation
, **, ns = significant at 0.01, 0.0001 and not significant, respectively.

A3. ANOVA table for the effect of GA_{4+7} on the yield of French beans

MEAN SUM OF SQUARES

Source of variation	df	Experiment 1	Experiment 2
Block	3	78.31 ***	14.83 ns
Treatment	4	440.78 ****	171.66 ****
Error	12	6.96	12.06
Corrected total	19	2081.62	875.904
CV		1.69	2.16

df = degrees of freedom
CV = % coefficient of variation
, *, ns = significant at 0.001, 0.0001 and not significant, respectively.

A4.ANOVA table for the effect of Accel on pod length of French beans

MEAN SUM OF SQUARES

Source of variation	df	Experiment 1	Experiment 2
Block	3	0.30***	0.45
Treatment	4	0.35***	0.54**
Error	12	0.034	0.61
Corrected total	19	2.69	4.22
CV		1.65	1.82

df = degrees of freedom
CV = % coefficient of variation
***, ns = significant at 0.001 and not significant, respectively.

A5. ANOVA table for the effect of Accel on pod number of French beans

		MEAN SUM OF SQUARES	
Source of variation	df	Experiment 1	Experiment 2
Block	3	8.99*	17.06*
Treatment	4	47.34****	79.82****
Error	12	2.44	4.99
Corrected total	19	245.58	430.31
CV		2.55	3.56

df = degrees of freedom
CV = % coefficient of variation
*, ****, ns = significant at 0.05 and 0.0001, respectively.

A6. ANOVA table for the effect of Accel on the yield of French beans

		MEAN SUM OF SQUARES	
Source of variation	df	Experiment 1	Experiment 2
Block	3	2.69ns	1.34ns
Treatment	4	48.38****	14.65****
Error	12	1.03	1.01
Corrected total	19	213.89	74.75
CV		2.54	2.46

df = degrees of freedom
CV = % coefficient of variation
****, ns = significant at 0.0001 and not significant, respectively.

A7. ANOVA table for the effect of BA on pod length of French beans

MEAN SUM OF SQUARES

Source of variation	df	Experiment 1	Experiment 2
Block	3	0.018ns	0.07ns
Treatment	4	0.028ns	0.11ns
Error	12	0.12	0.10
Corrected total	19	1.63	1.84
CV		386.06	-446.16

df = degrees of freedom
CV = % coefficient of variation
ns = not significant

A8. ANOVA table for the effect of BA on pod number of French beans

MEAN SUM OF SQUARES

Source of variation	df	Experiment 1	Experiment 2
Block	3	0.98ns	1.40ns
Treatment	4	2.86ns	10.52ns
Error	12	2.06	4.91
Corrected total	19	39.09	105.25
CV		598.09	234.54

df = degrees of freedom
CV = % coefficient of variation
ns = not significant.

A9. ANOVA table for the effect of BA on pod yield of French beans

MEAN SUM OF SQUARES

Source of variation	df	Experiment 1	Experiment 2
Block	3	2.25ns	0.40ns
Treatment	4	3.29ns	0.92ns
Error	12	1.46	1.22
Corrected total	19	37.41	19.52
CV		129.85	182.06

df = degrees of freedom
CV = % coefficient of variation
ns = not significant

A10. ANOVA table for the effect of GA_{4+7} on chlorophyll content of French bean leaves

MEAN SUM OF SQUARES

Source of variation	df	Experiment 1	Experiment 2
Block	3	0.02ns	0.11***
Treatment	4	0.02ns	0.02ns
Error	12	0.02	0.01
Corrected total	19	0.35	0.51
CV		8.34	5.71

df = degrees of freedom
CV = % coefficient of variation
***, ns = significant at 0.001 and not significant, respectively.

A11. ANOVA table for the effect of GA_{4+7} on chlorophyll content of French bean pods

MEAN SUM OF SQUARES

Source of variation	df	Experiment 1	Experiment 2
Block	3	0.001ns	0.01ns
Treatment	4	0.005ns	0.01ns
Error	12	0.03	0.003
Corrected total	19	0.06	0.10
CV		11.06	12.46

df = degrees of freedom
CV = % coefficient of variation
ns = not significant, respectively.

A12. ANOVA table for the effect of GA_{4+7} on chlorophyll retention by French bean pods

MEAN SUM OF SQUARES

Source of variation	df	Experiment 1	Experiment 2
Block	3	0.001ns	0.01**
Treatment	4	0.01*	0.005ns
Error	12	0.002	0.002
Corrected total	19	0.05	0.07
CV		15.11	17.66

df = degrees of freedom
CV = % coefficient of variation
*, **, ns = significant at 0.05, 0.01 and not significant, respectively.

A13. ANOVA table for the effect of Accel on chlorophyll content of French bean leaves

MEAN SUM OF SQUARES

Source of variation	df	Experiment 1	Experiment 2
Block	3	0.03ns	0.06***
Treatment	4	0.05**	0.03**
Error	12	0.01	0.01
Corrected total	19	0.40	0.37
CV		5.96	5.16

df = degrees of freedom
CV = % coefficient of variation
, *, ns = significant at 0.01, 0.001 and not significant, respectively.

A14. ANOVA table for the effect of Accel on chlorophyll content of French bean pods

MEAN SUM OF SQUARES

Source of variation	df	Experiment 1	Experiment 2
Block	3	0.01ns	0.001ns
Treatment	4	0.03***	0.04***
Error	12	0.04	0.01
Corrected total	19	0.18	0.24
CV		1.56	12.96

df = degrees of freedom
CV = % coefficient of variation
***, ns = significant at 0.001 and not significant, respectively.

A15. ANOVA table for the effect of Accel on chlorophyll retention by French bean pods

MEAN SUM OF SQUARES

Source of variation	df	Experiment 1	Experiment 2
Block	3	0.001ns	0.01ns
Treatment	4	0.03****	0.04****
Error	12	0.002	0.003
Corrected total	19	0.15	0.20
CV		12.38	15.72

df = degrees of freedom
CV = % coefficient of variation
****, ns = significant at 0.0001 and not significant, respectively.

A16. ANOVA table for the effect of GA_{4+7} on Water Content (percent) of French beans pods

MEAN SUM OF SQUARES

Source of variation	df	Experiment 1	Experiment 2
Block	3	3.43ns	1.40ns
Treatment	4	44.17****	16.80***
Error	70	3.23	2.95
Corrected total	77	486.80	229.20
CV		2.02	1.94

df = degrees of freedom
CV = % coefficient of variation
, *, ns = significant at 0.001, 0.0001 and not significant, respectively.

A17. ANOVA table for the effect of Accel on Water Content (percent) of French beans pods

		MEAN SUM OF SQUARES	
Source of variation	df	Experiment 1	Experiment 2
Block	3	3.68ns	0.25ns
Treatment	4	26.33****	26.09****
Error	70	2.32	1.96
Corrected total	77	299.89	243.19
CV		1.72	1.59

df = degrees of freedom
CV = % coefficient of variation
, *, ns = significant at 0.001, 0.0001 and not significant, respectively.

A18. ANOVA table for the effect of BA on Water Content (percent) of French bean pods

Source of variation	df	Experiment 1	Experiment 2
Block	3	2.58ns	2.28ns
Treatment	4	6.83ns	14.14**
Error	70	3.63	3.92
Corrected total	77	305.89	344.39
CV		-411.87	-2262.95

df = degrees of freedom
CV = % coefficient of variation
*, **, ns = significant at P = 0.05, 0.01 and not significant, respectively.

A19. ANOVA table for the effect of GA_{4+7} on wilting of French beans pods

		MEAN SUM OF SQUARES	
Source of variation	df	Experiment 1	Experiment 2
Block	3	11.55ns	24.38****
Treatment	4	9.22ns	46.17****
Error	70	8.72	3.38
Corrected total	77	2292.75	937.49
CV		10.99	8.08

df = degrees of freedom
CV = % coefficient of variation
*, ****, ns = significant at 0.05, 0.0001 and not significant, respectively.

A20. ANOVA table for the effect of Accel on wilting of French beans pods

		MEAN SUM OF SQUARES	
Source of variation	df	Experiment 1	Experiment 2
Block	3	6.08ns	35.68****
Treatment	4	14.39ns	41.61****
Error	70	6.22	4.72
Corrected total	77	1145.39	1871.95
CV		10.65	8.35

df = degrees of freedom
CV = % coefficient of variation
****, ns = significant at 0.0001 and not significant, respectively.

A21. ANOVA table for the effect of GA_{4+7} and Accel on chilling of French beans pods

MEAN SUM OF SQUARES

Source of variation	df	GA_{4+7}	Accel
Block	3	22.45ns	0.91ns
Treatment	4	1.08ns	1.44ns
Error	70	9.16	9.68
Corrected total	77	799.39	757.69
CV		13.14	13.86

df = degrees of freedom
CV = % coefficient of variation
, *, ns = significant at 0.01, 0.001 and not significant, respectively.

A22. ANOVA table for the effect of GA_{4+7} on the rate of water loss by French beans pods (Experiment 1)

MEAN SUM OF SQUARES

Source of variation	df	1	2	3	4	5	6	7
Block	3	4.37ns	3.97ns	2.79ns	4.84ns	4.39ns	20.34ns	30.06*
Treatment	4	15.94***	33.58****	44.43***	50.00***	84.28****	99.05****	161.09****
Error	70	3.43	5.81	8.59	11.99	13.42	16.05	9.63
Corrected total	77		610.30	927.26	1109.74	1325.85	1601.62	1464.30
CV			22.09	20.69	19.58	17.20	16.10	10.66

df = degrees of freedom
CV = % coefficient of variation
*, **, ***, ****, ns = significant at 0.05, 0.01, 0.001, 0.0001 and not significant, respectively.

A23. ANOVA table for the effect of Accel on water loss by French bean pods (Experiment 1)

		MEAN SUM OF SQUARES						
Source of variation	df	1	2	3	4	5	6	7
Block	3	2.99ns	1.78*	5.71ns	4.32ns	18.21ns	19.68ns	12.76ns
Treatment	4	7.40ns	24.04**	27.46*	28.03ns	47.75*	59.88**	130.71****
Error	70	3.60	7.07	8.47	13.41	14.43	16.54	12.82
Corrected total	77	341.27	599.55	771.00	1097.74	1303.76	1486.29	1535.57
CV		31.80	24.17	19.92	20.04	17.24	15.74	11.66

df = degrees of freedom

CV = %-coefficient of variation

*, **, ***, ****, ns = significant at 0.05, 0.01, 0.001, 0.0001 and not significant, respectively.

A 24. Effect of BA on the rate of water loss by French bean pods (Experiment 1)

MEAN SUM OF SQUARES

Source of variation	df	1	2	3	4	5	6	7
Block	3	0.62	1.05ns	3.57ns	4.88ns	7.45ns	15.15ns	16.40ns
Treatment	4	4.12	10.03ns	10.76ns	8.22ns	22.85ns	13.22ns	31.88*
Error	70	5.20	7.50	8.89	8.38	12.79	15.34	12.26
Corrected total	79	434.79	619.03	725.82	668.38	1019.87	1201.05	1049.32
CV		1753.50	3040.66	683.32	491.69	351.97	407.39	217.35

df = degrees of freedom

CV = % coefficient of variation

*, **, ns = significant at 0.05, 0.01 and not significant, respectively.

A25. ANOVA table for the effect of GA_{4+7} on water loss by French beans pods (Experiment 2)

		MEAN SUM OF SQUARES						
Source of variation	df	1	2	3	4	5	6	7
Block	3	5.07ns	2.58ns	0.90ns	2.33ns	5.00ns	13.13ns	18.96ns
Treatment	4	9.33**	24.50***	19.51***	20.99*	37.88**	62.42***	91.50**
Error	70	2.27	4.40	4.45	7.46	10.76	14.66	25.64
Corrected total	77	227.81	472.39	448.02	731.73	1206.48	1718.46	2871.91
CV		25.44	18.60	13.28	13.66	13.53	13.52	15.17

df = degrees of freedom

CV = % coefficient of variation

*, **, ***, ****, ns = significant at 0.05, 0.01, 0.001, 0.0001 and not significant, respectively.

A26. ANOVA table for the effect of Accel on water loss by French beans pods (Experiment 2)

MEAN SUM OF SQUARES

Source of variation	df	1	2	3	4	5	6	7
Block	3	0.64ns	3.15ns	1.94ns	7.74ns	11.42ns	19.64ns	34.26ns
Treatment	4	6.38*	20.79***	23.10**	19.24ns	38.28*	65.27*	122.09**
Error	70	2.28	4.20	6.33	10.44	15.08	20.56	32.96
Corrected total	77	201.62	455.82	635.41	1093.69	1818.82	2824.68	4408.10
CV		24.89	17.51	15.34	15.58	15.48	15.49	16.78

df = degrees of freedom

CV = % coefficient of variation

*, **, ***, ****, ns = significant at 0.05, 0.01, 0.001, 0.0001 and not significant, respectively.

A27. Effect of BA on the rate of water loss by French bean pods (Experiment 2)

MEAN SUM OF SQUARES

Source of variation	df	1	2	3	4	5	6	7
Block	3	4.13ns	1.84	5.18ns	10.88ns	7.43ns	14.99ns	31.25ns
Treatment	4	1.46ns	3.13	5.22ns	6.77ns	11.22ns	19.55ns	29.21ns
Error	70	3.46	6.34	6.92	9.06	9.66	11.69	19.15
Corrected total	77	261.72	463.56	531.59	734.79	795.11	1109.10	1758.33
CV		1294.72	574.06	506.02	385.86	371.19	357.07	525.70

df = degrees of freedom

CV = % coefficient of variation

*, **, ns = significant at 0.05, 0.01 and not significant, respectively.

A28. Effect of GA_{4+7} on post storage water loss by French bean pods

MEAN SUM OF SQUARES

Source of variation	df	1	2	3	4
Block	3	11.02***	56.55***	83.67***	112.88***
Treatment	4	0.84ns	18.71ns	25.03ns	24.62ns
Error	70	1.95	11.22	17.21	21.3
Corrected total	77	453.50	2555.16	3743.87	4905.59
CV		41.04	42.69	35.7	29.07

df = degrees of freedom
CV = % coefficient of variation
*, **, ***, ****, ns = significant at 0.05, 0.01, 0.001, 0.0001 and not significant, respectively.

A29. Effect of Accel on post storage water loss by French bean pods

MEAN SUM OF SQUARES

Source of variation	df	1	2	3	4
Block	3	11.75**	47.35***	118.92****	153.68ns
Treatment	4	0.07ns	5.12ns	5.50ns	5.53ns
Error	70	3.01	8.72	16.33	21.41
Corrected total	77	494.37	2071.59	3517.72	5079.26
CV		50.7	37.72	33.85	28.31

df = degrees of freedom
CV = % coefficient of variation

*, **, ***, ****, ns = significant at 0.05, 0.01, 0.001, 0.0001 and not significant, respectively .