STUDIES ON THE EFFECT OF VAM AND GROWTH REGULATORS ON *Gladiolus grandiflorus* L. cv. Jessica.

By

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DISSERTATION SUBMITTED TO CHAUDHARY CHARAN SINGH HARYANA AGRICULTURAL UNIVERSITY, HISAR, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF:

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2002

Affectionately
Dedicated
To My
Beloved Parents

CERTIFICATE-I

This is to certify that this dissertation entitled "**Studies on the effect of VAM and growth regulators on *Gladiolus grandiflorus* L. cv. Jessica**", submitted for the degree of Doctor of Philosophy in the subject Horticulture of Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by Mr. Sunil Kumar under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE-II

This is to certify that this dissertation entitled" **Studies on the effect of VAM and growth regulators on *Gladiolus grandiflorus* L. cv. Jessica"**, submitted by Mr. Sunil Kumar to CCS Haryana Agricultural University, Hisar, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the subject of Horticulture has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an external examiner.

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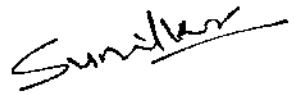

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CONTENTS

INTRODUCTION...

India is endowed with natural wealth of plant material, which are of great floricultural importance. Flowers are important in India mainly for aesthetic, social and economic considerations and consist of growing of annual, biennial and perennial plants either under glass or outdoors. The use of flowers in the gardens for the sake of their beautiful colours, forms and their fragrance cultivation of flowers, trees and shrubs is as old in India as the people themselves. The developing aesthetic taste and using cut flowers for decorating the home and surroundings is gaining importance and hence possessed much commercial importance including ornamental foliage plants for earning foreign exchange. Cut flowers constitute 45% share of the total world trade in floricultural product and India accounts for just 0.6% of the cut flower trade in world market (1990). The most important cut flowers are orchid, rose, carnation, chrysanthemum, gladiolus and some annual flowers. Now a days, demand of cut flowers being high in the world market, gladiolus is gaining importance as a cut flower and thereby earning foreign exchange during winter.

In India, flower cultivation has been confined largely to the fields around major cities and pilgrimage centers. However, some local and inter state trade in flower has also developed. The major cities under flower production are Tamil Nadu, Karnataka, West Bengal, Andhra Pradesh, Rajasthan and Maharastra and the intensive cut flower production has been taken up in areas concentrated around Bangalore, Trivendrum, Pune, Nasik and Delhi. Thus, in India comprises area under flower crops increased from 38000 hectare (National Horticultural Board, 1992-93) to 65,000ha. Among which 10,000 hectares are under modern floriculture.

However, with such large area under flower production, no organized effort has been made to develop it as floricultural Industry. Production of cut flower has now registered a steady increase in the recent past, but it has not kept pace with the demands of a large country like India. Besides this, according to a study conducted by APEDA (Agricultural and Processed Food Products Export Development Authority), the value of floricultural product is estimated to be around Rs.205 corers comprising transaction worth Rs.105 crores in the Traditional Sector and Rs.100 crores in Cut Flower Sector. Now a days it crossed over Rs. 250 crores with export exceed to more than 100 crores (APEDA, 1989).

Gladiolus is by far the best-suited bulbous plant in India and known as the queen of ornamental bulbous crops because it not only provides a wide range of flower colours but large number of florets per spike also. In the international cut flower trade, it occupies 4th place, next to the tulips in European Market. Gladiolus is very popular as cut flower and for display material in the gardens. It is also used in herbaceous borders, beddings, rockeries and pots.

Gladiolus, a genus of monocotyledonous plant, family Iridaceae and sub-family Ixioideae. Gladiolus is native of mediterranean region, tropical and South Africa and Mascarene Islands. The word gladiolus was coined by Pliny the Elder (AD 23-79) and has been derived from Latin word "Gladius" owing to sword shape leaves. Gladiolus is also named as Iris, Xiphion (according to Ancient Greeks) and popularly known as "Sword Lilly".

Gladiolus has potential for export as a cut flower besides having aesthetic value in the garden and is very much useful for the purpose of room decoration and various types of functions and ceremonies. Developing the techniques for growing it winter season under subtropical condition for better flowerings as well as corms and cormel production is to be established.

The use of VAM for the cut flower, corm and cormel production is a recent advances and unique of its kind. VAM (Nutrilink) plays an important role in increasing the plant growth and health by improving availability of soil phosphorus and trace elements such as Zn, Cu etc., enhances the uptake of applied fertilizer nitrogen, improves root proliferation and reduces symptoms incited by disease producing organisms. Also it utilize the insoluble soil phosphate and greatly increases the phosphorus uptake by a better exploration of soil beyond the depletion zone and by hyphal proliferation in favourable micro environment and extremely advantageous to crops grown in low fertility soils which are characteristics of poorly managed, continuously cropped agricultural lands as well as drastically disturbed landscape and mined reclamation sites. Thus, increase in mineral uptake as the result of mycorrhizal associations reflects increase plant survival, growth and yield as well as nutrition.

Growth regulators like GA_3 and Kinetin helps in breaking dormancy, retards the senescence and stimulates the synthesis of specific RNA and also helps in protein metabolism. It is seen that during senescence DNA, RNA, protein, enzyme activity, starch content decreases while sugar content increases.

Cut flowers are living actively metabolizing plant part subject to the same basic aging phenomena as are entire plant. Since they are cut off from their natural sources of raw material for metabolic functioning, efforts to supply these necessities for metabolic seldom completely successful. Flowers removed from the plants routinely deteriorate much more quickly than other flowers left on the plants under similar environmental conditions. Petals are an excellent model system for the study of fundamental senescence processes. If flowers are to provide their longest possible decorative role, controlled rate of opening or their development is needed as well as maintenance of colour stability.

Term 'Vase Life' represents the potential useful longevity of the flower at the final consumer's home. The points of termination of vase-life vary from the first sign of wilting or fading to the total death of all flowers with all the intermediate values between the points. In addition to vase-life, other characteristics are also important in evaluation of post harvest quality viz. Final flower size and shape, changes in flower fresh weight, floret development in spikes and development of lateral florets, turgidity and freshness of flowers up to reaching to consumer, objective measurement of changes in petal colour, stability and strength of the stem or pedicel, foliage or stem yellowing or browning.

Basically, there are two main differences between cut-flowers and agricultural products viz. Cut-flower is a more complex organ than seeds, fruits and vegetables because inflorescence is composed of many morphological units including sepal, petal, androcoeium, gynoecium, stem and often leaves. Stem plugging is one of the main factors determining longevity. Also, the complex nature of cut-flowers require special attention in developing handling technique, concentration of sugar and other substances used in solution for 'Pulsing' and 'Bud-opening' of flower is determined. Secondly cut flowers possess two distinct stages in the physiology of the flowers. The first stage is of bud growth and development of the plant to full opening. The second stage is of maturation, senescence and wilting. Handling technique to achieve cut-flower longevity must achieve two seemingly conflicting purposes, promotion of growth process in the first stage and retardation of metabolic processes leading to senescence at the second stages.

In gladiolus, spike length, number, size and colour of the floret are some of the characters used for determining the quality. The rachis should be strong and straight without any branching, floret should be uniformly spaced along the stem, open slowly and face in one direction. For this, cutting of spike should be done at the tight bud

stage with at least four leaves left on plant and from one to five buds showing colour.

Since indiscriminate use of fertilizers had led several problems viz. Pollution of ground water and streams. Therefore, new resources for increasing crop productivity need to be explored. For this, a more intensive investigation into the plant-soil-microflora complex is needed. But there is limited information on the role of mycorrhizal fungi in horticultural crop production. Most of the information on use of mycorrhizae are associated with family Orchidaceae, Ericaceae, Asteraceae, Rosaceae for their identification and role for soil amendment, water relation, stress tolerance etc. but regarding the family Iridaceae in which gladiolus belong, no work has been done about the identification of suitable strain and their role of increasing crop productivity have been done so far.

Therefore, the present investigation entitled, **"Studies on the effect of VAM and growth regulators on *Gladiolus grandiflorus* L. cv. Jessica"** was carried out at horticultural farm, Department of Horticulture, CCS Haryana Agricultural University, Hisar during year 1999-2000 and 2000-2001 with the following **objectives:**

1. To investigate the effect of VAM and growth regulators on vegetative growth, flowering and corm production in gladiolus.
2. To study the effect of presoaking and foliar spray of growth regulators on vegetative and reproductive parameters.
3. To gain the knowledge about the various physiological and bio-chemical changes during vase-life.
4. Longevity/vase-life

REVIEW OF LITERATURE...

The use of growth regulator and bio-fertilizer is developing science and has a great promise in the modern agriculture technology. A good number of growth regulators are now available in the market that can be used effectively and efficiently in different ornamentals crops both for the traditional flower production as well as cut flower trade. Results of experiments on growth, flowering and corms parameters, physiological and bio-chemical changes during vase-life, and ethylene and carbon dioxide evolution in gladiolus have revealed that several growth regulators are very effective. Though precise information is still meager concerning the part played by kinetin in the retardation of the bio-chemical changes associated with senescence but it is clear that they must exert a control through some fundamental cellular processes. The results provide evidence that kinetin may control senescence of plant parts by substituting in some way for an essential factor necessary for maintaining the synthesis of RNA. Evidently, the hormone needed to retard senescence is not the same for all species, for an auxin will not retard the leaf senescence in Xanthium (Osborne, 1965) and kinetin is ineffective when supplied to *Prunus* (Sugiura *et al.*, 1962). So far, however, little information concerning the regulation of bio-chemical processes of senescence by another major group of endogenous hormones, the gibberellins. Also, no analysis of the physiological or bio-chemical effects has been made. In this chapter, an attempt has been made; a brief resume of the literature is reviewed under the following headings.

EFFECT OF VESICULAR ARBUSCULAR MYCORRHIZA (VAM) ON GROWTH CHARACTERS OF *Gladiolus grandiflorus* L. cv. Jessica.

Mycorrhiza literally means 'Fungus Root'. In 1885, Frank coined the term Mycorrhizae to describe the symbiotic association of plant roots and fungi. In 1879, Anton De Bary introduced the concept of symbiosis or 'living together'. V.A.M. is a endomycorrhizal fungi and hyphae generally penetrate through the epidermis or root hair into the cortical cells.The penetrating hyphae after form specialized structure called vesicles and arbuscules.

Since knowledge of endomycorrhizal fungi is generally lacking, but it seems probable that for a particular purpose some fungi will prove to be superior to others. Also, there is limited information on the role of mycorrhizal fungi in the horticultural crop productions.

Vesicular- arbuscular mycorrhizal (V.A.M.) fungi can form a symbiosis with a wide variety of plant hosts. The symbiosis stimulates the growth and development of plants (Biermann and Lindermann, 1983; Crews and Johnson, 1978; Plenchette *et al.*, 1983) increases absorption of plant immobile elements (Ames *et al.*, 1983; Mosse and Haymen, 1973), increases drought tolerance (Auge *et al.*, 1986; Bolgiano *et al.*, 1983) and reduces disease incidence (Dehne, 1982). Moreover, colonization with VAM fungi increases uniformity and reduces mortality.

Wang *et al.* (1993) observed increased survival rates of gerbera and nephrolepis plantlets inoculated with VAM after 8 weeks.

Crafts and Miller (1974) stated that VAM takes over cytokinin synthesis or high indigenous concentration of cytokinins in the roots, modifies growth and predisposes the root to mycorrhizal infection.

Crews *et al.* (1978) obtained enhanced growth of three woody ornamentals viz. *Viburnum suspension*, *Podocarpus macrophylla* and *Pittosporum tobira* with inoculation with two endomycorrhizal fungi.

Vanderploeg *et al.* (1972) observed superior growth of two lilies (*Lilium sp.*) in a medium containing apparently unsterilized soil mixed soil from beneath a naturally inoculated garden.

Johnson and Crews (1979) obtained better survival and growth four months after transplanting with *Glomus mossae* inoculated azalea (*Rhododendron simsii*) plants than with non-mycorrhizal plants transplanted to a normal (nonsterilized) site.

Mosse *et al.* (1969) showed that onions inoculated with VA-Mycorrhizal fungi grew better than non-inoculate plants in unsterilized soils.

Soroa *et al.* (1998) observed improved plant development, when seeds of china aster were inoculated with *Glomus mossae* by pelleting the seeds and also showed *G. versiculiferum*, *G. versiformae* and *S. pellucida* produced the best result.

Busch and Lelly (1997) found that plants viz. *Dianthus deltoids, Sedum album, Festuca ovina* grown in mycorrhizal soil exhibited better growth than control plant. However, seedling of *Petunia x hybrida* inoculated with arbuscular mycorrhizal fungi (AMF) after 35 days showed plant height; fresh weight and root length were not significantly different from the control plant. Though the micro bio-fertilizer improved plant height, fresh weight, number of leaves and root length (Wang and Ling, 1997).

2.2 EFFECT OF VESICULAR ARBUSCULAR MYCORRHIZA (VAM) ON FLOWERING CHARACTERS OF *Gladiolus grandiflorus* L. cv. Jessica.

Wang *et al.* (1993) observed VAM inoculated gerbera plants produced larger and more flowers than non-inoculated gerbera, whereas there were no differences between stem length (141 to 145 mm) and diameter (64 to 65 mm) related to treatments.

Daft and Okusanya (1973) observed that mycorrhizal inoculation increased the numbers of petunia flowers produced per plant, whereas Johnson *et al.* (1982) reported that mycorrhizal inoculation in Chrysanthmum increased flower dry weight.

Bagyaraj and Powell (1985) opined mycorrhizal inoculation improved flower production as well as vegetative growth of marigold.

Plenchett *et al.* (1983) reported that mycorrhizal inoculation improved growth of marigold in a fumigated soil. However, Easter lily bulbs when inoculated with VA-Mycorrhizal fungi, growth measured in terms of bulb weight gain, root volume and total leaf area (Ames and Lindermann, 1978).

Chen and Chang (1996) stated that ornamental plants treated with VAM, improved plant growth of Scarlet Sage (*Salvia splendens*), Ageratum (*Ageratum hostonianum*), Wedelia (*Wedalia trilobata*) & Cigar flower (*Cuphea ignea*), showed increased number of flowers/plant in polyantha (*Primula polyantha*) and early flowering of scarlet sage and star cluster (*Pentas Lanceolata*).

The effects of soil inoculation with *Glomus etunicatum* on the growth and flowering of *T. erecta* and *Z. elegans* seedlings were studied and

observed mycorrizal inoculation increased vegetative growth parameters and the number of flowers per plant compared with control (Aboul-Nasr, 1994).

2.3 EFFECT OF GROWTH REGULATORS ON GROWTH CHRACTERS OF *Gladiolus grandiflorus* L. cv. Jessica.

Pre soaking treatment with chemical proved effective in growth and flowering of a number of bulbous ornamentals.

Treatments with GA_3 are known to promote corm and cormel sprouting (Mukhopadhya & Banker, 1986; Misra & Singh, 1989).

Leena *et. al.* (1992) reported significant influence on the height of the plants, both at six weeks and at eight weeks after planning of gladiolus cv. Friendship and observed GA_3 at 100 ppm gave the maximum height to the plants, influenced the number of leaves and total leaf area was also significant.

Das *et al.* (1992) revealed that foliar application of GA_3 at 200 ppm, once 30 days after planting and second 50 days after planting in *Hemerocallis aurantica* bulbs produced maximum plant height, which was significantly greater than the control. Also showed increased leaf number over the control.

Increased plant height with GA_3 at 1000 ppm in *Lilium longiflorum* was reported by De Hertogh and Blackely (1972) whereas, Mukhopadhyay and Sadhu (1985) observed pre plant soaking of tuberose bulbs with GA_3 at 100 ppm reduced plant height, while Bhattacharjee and Mukherjee (1975) noticed tuberose bulbs treated with GA_3 produced more number of leaves per plant.

Roychoudhuri *et al.* (1985) reported that pre soaking of corms of gladiolus cv. Psittacinus hybrid in solution of GA_3 at 100 ppm and Kinetin at 25 ppm were effective in increasing the leaf number, whereas Kinetin at 25 ppm increased the plant height by about 28.84 per cent compared to control and marked improvement in the weight of cormlets was observed by treating with Kinetin at both the concentration viz., 25 and 50 ppm. However, GA_3 at 50 ppm increased the stalk length but adversely affected the diameter of flowers.

Mukhopadhyay and Bankar (1986) opined that soaking gladiolus cv. Friendship corms with GA_3 in the dark for 24 hours advanced sprouting of corms (10 and 25 ppm) and also improved plant height irrespective of the concentration used, while Tonecki (1979) reported stimulation of corms sprouting following GA_3 treatment, but Ginzburg (1974) noted invitation in cormel germination.

An investigation was undertaken to study the growth and flowering response of Chrysanthmum cv. Co-1 to the foliar treatments of GA3 at 50, 100 and 150 ppm, highest plant height with GA at 150 ppm and significant increased in number of leaves by the treatment with all concentration were recorded (Dutta and Ramadas, 1998). Similar effect of GA on chrysanthmum by Fukuda *et al.* (1987) and Nagarjuna *et al.* (1988) and on Dahlia by Bhattacharjee (1984) on plant growth was proved.

Bhattacharjee (1984) studied the effect of soil drench application of GA_3 (10,100,1000 ppm each) in gladiolus cv. Friendship and reported that GA_3 at 10 or 100 ppm increased vegetative growth. On the other hand, Dua *et al.* (1984) observed that most of the growth, flowering and corm production parameters the spraying of GA_3 at 100 ppm on cv. Sylvia crop was most effective treatments.

Patil (1991) observed that soaking of corms of cvs. Joselita and Sylvia with 100 ppm GA_3 gave early sprouting of corms, whereas pre-planting soaking of gladiolus corms with GA_3 at 50 or 100 ppm resulted in higher plant height and leaf area index (Kumaran, 1993).

Tonecki (1979) revealed that gladiolus corms soaked for 24hours in GA_3 or Kinetin each at 100, 500, 1000 and 2000 ppm prior to planting stimulated corms sprouting except Kinetin, which inhibited early growth. However, GA_3 hastened differentiation of floral primordial but kinetin retarded corm sprouting and shoot apex differentiation when gladiolus corms soaked for 24hours in GA_3 or kinetin each at 100, 500, 1000 and 2000 ppm (Tonecki, 1980).

Pathak *et al.* (1980) opined that pre-treatment of the tuberose bulbs with GA_3 (300 ppm) had no effect while kinetin (300 ppm) appreciably stimulated sprouting and also observed that GA_3 and kinetin inhibited flowering.

Choudhary (1987) observed application of GA_3 (50-100 ppm) to the tuberose plant markedly increased plant height and the number of leaves per plant. Rhizomes of tuberose soaked in GA_3 (200 ppm) solution for six hours improved plant growth (Dhua *et al.*, 1987)

Poliantus tuberosa cv. Single, bulbs were dipped in solution of GA_3 (100 or 200 ppm) for 24hours or plants were treated with foliar application at 40 days after planting. GA_3 at 200 ppm enhanced bulb sprouting and decreased the number of days required for sprouting from 20.83 in the control to 12.03, however, foliar application of 200 ppm GA_3 significantly increased plant height (Davendra *et al.*, 1999).

Mishra *et al.* (1996) investigated the implication of GA_3 spraying on the standing crops of gladiolus cv. Sylvia and observed GA_3 application as a single foliar spray at 53 days after planting corms enhanced vegetative growth, flowering and number of corms and cormels produced, but adversely affects individual corm weight and duration of the whole spike at GA 200 or 400 ppm. They also concluded that apart from corm size, GA_3 at 100 and 200 ppm gave encouraging result.

Arora *et al.* (1992) found that gladiolus corms dipped for 24h in the solution of GA_3 at 100 ppm accelerated sprouting of cormels by 4.6, 3.2 and 4.8 days in cv. Aldebaran, Pusa Suhagin and Mayur, respectively.

Talukdar and Paswan (1996) stated that spray chrysanthemum cv. Prof. Harris when sprayed with GA_3 at 10, 20 and 40 ppm, significantly increased plant height and largest flower were obtained with 40 ppm GA_3. However, re-soaking of seeds in GA_3 and kinetin accelerated seed aging of *Helichrysum bracteatum* and extended seed aging due to increased leakage of electrolytes and decreased germination rate (Grzesik, 1992).

2.4 EFFECT OF GROWTH REGULATORS ON FLOWERING CHARACTERS OF *Gladiolus grandiflorus* L. cv. Jessica.

Gibberellic Acid showed increased growth, flowering, floret size and number of florets per spike in gladiolus (Auge, 1982; Bhattacharjee, 1984 and Dua *et al.*, 1984).

Leena *et al.* (1992) observed earliest flowering in GA_3 100 ppm treated corms cv. Friendship, whereas duration from spike emergence to opening did not differ significantly but the total duration of plant was the maximum (83.00 days), though lengthen the life of spikes. They also found the spike having maximum length (75.4 cm) and number of floret per spike (16.00) were produced by GA_3 at 50 ppm and was at par with GA_3 100 ppm (74.20 cm) and (15.00), respectively.

Das *et al.* (1992) reported appreciable increase in the length of flower spike and increased number of florets per spike with foliar application of GA_3 at 200 ppm than the control. However, pre-plant bulb soaking with GA_3 at 200 ppm increased the length of spike and flower yield in tuberose (Dhua *et al.*1987), increased rachis length of tuberose was obtained by Mukhopadhyay and Sadhu (1985) by pre-plant bulb treatment with GA_3 at 100 ppm and increased flower number in Hippeastrum was obtained by Bose *et al.* (1980).

Mukhopadhyay and Bankar(1986) found that soaking gladiolus cv. Friendship with GA_3 in the dark for 24hours, accelerated flowering (10 or 50 ppm) and improved spike length irrespective of the concentration used.

Tonecki (1980) studied the effect of GA_3 on flowering and concluded the growth substances play a significant role in an integration of developmental. process in gladiolus and Ginzburg (1974) confirmed GA_3 responsible for assimilate movement towards the inflorescence at the expense of the gladiolus corm, which might have influenced early emergence of flower spike. However, Gilbertson-Ferriss and Wilkins (1981) stated soaking of freshly harvested *Fressia hybrida* cv. Moya corms in solution of GA_3 for 30 minutes, did not decreased the time required for shoot emergence and days to flower and flower quality were not influenced also.

Wilfret and Roulston (1975) observed single application of 500 ppm GA_3 on Iceberg and Midnight Blue statice made at 87, 101 and 115 days after seeding reduced mean flowering time 92, 72 an 45 days, respectively and they also reported repeated spray were not beneficial and in some cases, delayed flowering as compared to single application.

Henny (1980) revealed that *Diffenbachia maculata* cv. Perfection sprayed with GA_3 in September, flowered within 105 days or less and

inflorescence number per plant was greater at 500 and 1000 ppm than at 250 ppm treatment. However, GA_3 treatment in cyclamen cultivars accelerated flowering 28 to 35 days and increased simultaneous flower production per plant upto 100 per cent. Leaf size, leaf count and plant size were not altered by GA_3. But, a single spray of 50 ppm GA_3 applied 60 to 75 days prior to desired time of bloom is suggested to promote more accurate, efficient scheduling of cyclamen crops (Widmer *et al.*,1974).

Harbough and Wilfret (1979) found caladium flower production can be increased by soaking tubers in 250 ppm GA_3 for 8 to 16h. However, foliar application of GA_3 at 250 and 100 ppm promoted flowering in a number of edible aroids viz. Tannia (*Xanthosoma sagittifolium*) and dasheen(*Colocasia esculenta*) Alamu and McDavid,1978.

Treatment with 500 or 100 ppm GA_3 plus 100 ppm kinetin applied at 4-days intervals hastened flowering of azalea, whereas, kinetin alone did not hasten flowering and a synergistic action of kinetin with GA_3 was observed by Furuta *et al.*, 1965).

An investigation was undertaken to study the growth and flowering response of chrysanthemum cv.CO.1 to the foliar treatments of GA_3 at 50, 100 and 150 ppm, flowering was advanced by GA_3, while extension of flowering duration coupled with improvement in flower quality were achieved from treatments with all concentration. Also, plants receiving GA_3 spray at 50 ppm produced flowers with longest flowering duration (Dutta and Ramadas, 1998). They also indicated that GA_3 treatments significantly advanced the formation flower buds and commencement of flowering and largest flower size (diameter) coupled with largest stalk in plants with GA_3 at 150 ppm.

Bhattachargee (1984) studied the effect of soil drench application of GA_3 (10, 100 and 1000 ppm each) in cv. Friendship and reported the GA_3 at 10 or 100 ppm stimulated spike and rachis length, accelerated floret size and number per spike and lengthened the duration of flowering.

Mukhopadhyay and Banker (1981) reported that soaking (24hours) of cv. Friendship corms in GA_3 advanced sprouting of corms (10 or 25 ppm) and accelerated flowering, plant height and spike length. On the other hand, treatment of corms with kinetin (25 or 50 ppm) increased floret size and number of florets per spike.

Mohanty *et al.* (1994) revealed that soaking (24hours) of cv. Vink's Beauty corms in 250 ppm of GA_3 concentration had significantly induced emergence of spike and subsequently colour break, increased more number of florets per spike, duration of flowering and weight of daughter corms.

Mahesh and Misra (1993) studied the effect of corm dipping (3hours) of cv. Snow Princess at the time of planting in aqueous solution of GA_3 at 200, 500 or 1000 ppm or spraying after 45 days of planting or dipping the corms at planting time and again spraying after 45 days of planting. They observed that GA_3 at 1000 ppm dipping + spraying induced earliest flowering, however GA_3 at 500 ppm as dipping alone proved best in increasing the floret diameter, weight of corms and rachis length, whereas number of florets per spike were maximum under 500 ppm GA_3 + spraying.

Dutta *et al.* (1998) studied the effect of GA_3 at 50, 100 and 150 ppm concentration on mums. GA_3 was sprayed after 30 and 45 days of planting and observed GA_3 spray had marked influence on growth and flowering. GA_3 at all concentration advanced formation of flower buds and commencement of flowering commence flowering (83.33 days) followed by $GA_3$100 ppm (92.33 days) and GA_3 50 ppm (99.67 days), decreased flowering duration with increase in its concentration and resulted in enhanced duration of flowering. Flower size, weight and length of stalk were also increased with GA_3 at 100 ppm.

Rak and Nowak (1989) investigated the effect of GA_3 on growth and flowering of snapdragon cutting and observed GA_3 sprayed at 100 ppm increased significantly the length of inflorescence and did not affect flower shape and colour.

Jana and Biswas (1982) observed the fewest days to flower opening when rhizomes of tuberose cv. Single were soaked for 24hours in solution containing GA_3 at 10 ppm.

Plants of the tuberose cv. Single were sprayed at 40 days after planting and twice more at fortnightly intervals with GA_3 at 25-100 ppm, increased spike length and number of florets per spike. Duration of flowering was improved with GA_3 at 100 ppm (Mukhopadhyay and Bankar, 1983). However, Biswas *et al.* (1983) revealed height number of flowers per spike

on plants sprayed with GA_3 (100 ppm) at 30 days after sprouting and again 20 days later.

Roychoudhury (1989) found that corms of gladiolus hybrid cv. Psittacinus soaked for 6hours in kinetin (25 or 50 ppm) before planting increased the number of florets per spike and flower size.

Karguzel *et al.* (1999) found that when gladiolus corms of cv, Eurovision were soaked in solution of 50 or 100 ppm GA_3 for 1h before planting, GA_3 at 100 ppm shortened the time from planting to harvest and increased flowering percentage, the length of spikes, the number of flowers per spike and diameter of flowers.

Ved *et al.* (1998) investigated the effect of GA_3 on the floral parameters of gladiolus cv. Friendship treated with GA_3 at 150 ppm followed by 100 ppm, improved time of flowering, spike length, floret length and number of florets per spike.

Polianthus tuberosa cv. Single, plants were treated with foliar application of GA_3 (100 or 200 ppm) at 40 days after planting and found GA_3 at 200 ppm significantly increased flower spike length, flower diameter and number of florets per spike (Devendra *et al.*,1999).Whereas, tuberose cv. Double, plants were treated with GA_3 at 200 ppm sustained early to flower and also showed increased spike length(Reddy *et al.*,1997).

Pal and Chowdhary (1998) elucidated soaking of gladiolus cv. Tropic Sea corms in aqueous solution of GA_3 (200 ppm) for 24h gave the greatest spike length (91.00 cm).

Preeti *et al.* (1997) investigated the effect of pre-planting treatment of bulbs of tuberose cv. Single with GA_3 (50, 100 and 200 ppm) and observed that GA_3 promoted the early appearance of flower spikes and promoted the number of flower spikes. However, treatment with GA_3 at 200 ppm produced the highest number of spikes with the longest length and the highest number of florets per spike.

Lee *et al.* (1999) found GA_3 (500 ppm) alone or in combination with BA (500 ppm) promoted seedling growth of *Lilium formelongi* cv. Neosan No. 2, while, flower stem length was promoted by GA_3 alone. Saniewski *et al.*, 1999, investigated stimulatory effect of GA_3 at 200 ppm on shoot growth and flowering of tulip cv. Gudoshnik and Apeldoorn.

Shi *et al.* (1997) observed application of GA_3 at 10 ppm to the freesia plants; the date of 30 per cent flowering was 70 days earlier than for the control.

A field experiment was conducted to evaluate the effects of GA_3 at 0, 100, 200, 300 ppm, which were applied as foliar spray or by soaking bulbs of narcissus for 24hours before planting and was found that application of GA_3 (300 ppm) in both methods gave the highest values for number of leaves per plant, number of inflorescence per plant, number of florets per inflorescence, length and fresh weight of both inflorescence and peduncle as well as the earliest flowering (El-Salllami, 1997).

Suh (1997) stated that days to flowering in tulip cv. Apeldoorn and Golden Apeldoorn were shortened by GA_3 injected into bulb scale tissues and total stem length was shortened after injection of GA_3 into bulb and flower bud compared with the control.

Shi *et al.* (1995) observed corms of freesia soaked in 10 or 40 ppm GA_3 before planting, showed the time to first flowering was 350 days earlier and time to 30 per cent flowering was 23 days earlier than the control.

Abo-ElGhait and Wahba (1994) investigated the response of *Viola odorat* to some growth regulators, 100-200 ppm GA_3 were sprayed in the field trial and observed the number of flowers per plant was highest with 200 ppm GA_3 but number of leaves and offsets were greatest with 100 ppm GA_3 and GA_3 at 200 ppm resulted more flower during the early part of the season.

Wrapping internodes in the cotton wick soaked in GA_3 to tulip stem cv. Gudoshnik at 250 ppm twice at 24h intervals, exogenous gibberellins markedly induced stem elongation and suggested GA_3 taken up by stem or synthesized under the influence of GA_3 induce elongation of internodes (Saniewski, 1999). However, gerbera cv. Terrasum, Liflora and Joyce treated with 100 ppm GA_3 at monthly intervals increased inflorescence diameter but had no effect on peduncle length (Farina *et al.*, 1989).

2.5 EFFECT OF GROWTH REGULATORS ON PHYSIOLOGICAL CHARACTERS OF *Gladiolus grandiflorus* L. cv. Jessica during vase-life.

The use of preservative solution to promote the quality and prolog the life of cut flowers has been known for many years and flower preservatives a composed mainly of sugar and germicides.

Senescence of flower petals is accompanied by visual characteristics changes in shape and colour (Halevy and Mayak, 1979). Since cellular membranes regulate many of the bio-chemical and bio-physical process taking place in the petals, very few studies have been conducted on senescence related changes. Senescence involves a predominance of catabolic process over anabolic process.

Rogers (1973) reported that flowers when cut and placed in water, they exhibit a changes in fresh weight. Typically, cut flowers initially increase and subsequently decrease in fresh weight. However, water uptake and water loss may fluctuate cyclically with an overall declining trend (Carpenter and Rasmussen, 1973; De Stigter, 1980,1981b).

Marousky (1968-69) revealed the gladiolus cv. White Friendship spike treated with 8-HQC at 600 ppm plus 4 per cent sucrose sustained a greater fresh weight, had more open florets and longer vase-life, increased water uptake and lowering of water loss through transpiration than spike held in water.

Lukaszewska (1978) studied the gladiolus cv. Oscar and Acca Laurentia, the flower spikes were cut when the first flower was opening and placed in various solution and observed solution containing 5 per cent sucrose or glucose and an antiseptic (HQC) chinosol improved flower opening and increases vase-life. Whereas, the flower of gladiolus cv. Dr. Magoe open more quickly in an aqueous solution of 600 ppm 8-HQC + 4 percent sucrose than water alone (Wilfret, 1983).

Nowak (1989) reported that gladiolus cut flower spikes cvs. Dukat, Lavenesque and Lastige Wittwe treated with a preservative solution consisting of 8-HQC at 200 ppm + GA_3 60 ppm + sucrose at 30g/l, markedly

improved bud opening, increased spike length, floret diameter and longevity of whole inflorescence.

Lal (1990) opined spikes of *Gladiolus primulinus* cv. Silver Horn were cut when the buds first showed colour and placed in solution of 8-HQC at 200 ppm resulted in the longest time to anthesis of the whole spike and exhibited the best flower quality for 15.70 days compared with 11.00 days for control.

Loss of water and the attendant decrease in fresh weight of petals is generally one of the most obvious symptoms of flower senescence (Halevy and Mayak, 1979) and increased water loss from petals is generally attributed to changes in cell membrane permeability (Borocho and Woodson, 1989).

Jones and Megan (1993) revealed HQC at 250 ppm increased vase-life of *Alstroemeria aurantca, Dandranthema grandiflorus* and *Dianthus barbatus* and improved longevity in *Freesia hybrida* cv. Aurora (Woodson, 1987) but had no effect on *Freesia hybrida* cv. Royal Crown (Accati-Garibaldi and Deambrogio, 1990).

The fresh weight was maintained at a significantly higher level in stems treated with HQC at 250 ppm in *Rosa hybrida* cv. Gabriella than the stems treated with water. Fresh weight of stems treated with HQC continued to increase upto 8 days after harvest, then declined. Whereas, there was no significant difference in fresh weight and solution uptake between *Dianthus caryophyllus* stems treated with HQC or water. Although fresh weight was maintained in stems treated with HQC for 7 days but no significant effect in vase-life (Jones and Megan,1993).

Mayak *et al.* (1970) reported decreased cytokinin level in rose petals during vase-life and inclusion of kinetin in the vase solution of leafless rose stems delayed flower wilting (Mayak and Halevy, 1974).

Dennis *et al.* (1983) observed post harvest flower fresh weight of *Zinnia elegans* increased when hold in solution containing 200 ppm 8-HQC and sucrose and decreased when held in deionized water and also found prolonged post-harvest decorative life of Zinnia flowers if held in a solution of 200 ppm 8-HQC + 1 per cent sucrose.

Garrod and Harris (1978) reported that application of GA_3 (200 ppm) to isolated carnation petals growing in agar or liquid medium delayed senescence. However, little or no effect on longevity by dipping carnation in GA_3 solution at 10 to 200 ppm (Nichols, 1968b). GA3 (100 to 400 ppm) in opening solution of carnation promoted opening but decreased longevity and caused discolouration of flowers (Cywinska-Smoter *et al.*, 1978).

Marousky (1971) evaluated bud cut mum flowers held in 8-HQC + sucrose increased more in fresh weight, absorbed more solution and developed into larger flowers than those held in water similar to that in roses and gladiolus.

Marousky and Raulston (1970) cut snapdragon in addition to chrysanthemum, rose and gladiolus and stated physiological response to 8-HQC + sucrose were similar to those of roses, gladioli and mum. Buds on snapdragon spikes held in 8-HQC + sucrose were similar to those or roses, gladioli and chrysanthemum. Buds on snapdragon spikes held in 8-HQC + sucrose developed into larger florets than buds on spikes held in water, also gained more weight and lasted longer and a pronounced feature of cut snapdragons was the tremendous increase in solution absorption when spikes were held in 8-HQC.

Addicot (1970) observed decrease in DNA, RNA, protein, enzyme activities and starch while increase in sugars and ethylene during senescence. Application of GA_3 and cytokinin are usually effective in retarding senescence and for growth stimulate the synthesis of specific RNA.

Gladioli held in solution containing 8-HQC had greater increase in fresh weight than the control, showing the benefit of the lower level of transpiration (Marousky,1968). Marousky (1972) observed sucrose in holding solution is undoubtedly important as an energy source, the inclusion of 8-HQC as an ingredient is also very important in maintaining rapid water uptake for extended periods of time.

Ramnuja Rao and Mohan Ram (1979) reported the spikes of gladiolus harvested at the green bud stage, GA_3 and sucrose caused a significantly greater percentage of flower openings when used together than individually.

Van Doorn (1998) revealed placing a daffodils (*Narcissus pseudonarcissus* L. cv. Carlton) flower in a vase with a rose (*Rosa hybrida* cv. Sonia) flower reduced water uptake by the rose and resulted in precocious wilting of its leaves and flower and in pedicel bending. Adding 8-HQC at 300 ppm to the vase solution overcame these symptoms and also HQC inhibited ethylene production and has antimicrobial properties.

The effects of GA_3 at concentration of 50 or 100 ppm on the vase-life of cut tuberose spikes were examined. GA_3 at 100 ppm were the most effective in improving the water uptake, maintaining the better water balance and thereby increasing the fresh weight of flowers which finally contributed to the increased vase-life (10.33 days) and increased number of florets which opened (56.89 per cent) per spike (Bhaskar and Rao, 1998).

Mayak and Halevy (1974) observed that kinetin at 60 ppm delayed the fading of cut roses (*Rosa hybrida* cv. Golden Wave) flower shoot, enhanced water uptake from the beginning, whereas promotion of water loss was observed only two days later. The consequence of the effects of kinetin on water uptake and loss resulted in a greater weight increment and observed kinetin treated flowers opened fully before wilting. Kinetin promoted the growth and expansion of the petals during vase-life and delayed in the weight decline in the tissues of the aging cut flowers. Thus, They inferred that kinetin has a dual effect on cut roses. At first it promotes petal growth and water uptake and later it delays processes associated with senescence and maintains petal turgidity for a longer period.

An investigation was undertaken to study the growth and flowering response of chrysanthemum cv. Co-1 to the foliar treatments of GA_3 at 50, 100 and 150 ppm and observed plants sprayed with GA_3 150 ppm produced flowers with the longest shelf life of 20 days, while a significantly greater improvement in shelf life of cut flowers was noted with all concentrations than the flowers from untreated plants enjoyed the shelf life of only 8.6 days.

Marousky (1968a)studied the physiological role of 8-HQC and sucrose in extending vase-life and improving quality of cut gladiolus flowers senesced, basal florets of spikes held in 8-HQC + sucrose lasted 63 days with 8.20 florets opened per spike at the time of senescence of basal floret and stems did not collapse or wilt after all florets had senesced than the

spikes held in water. He also found gladiolus held in 8-HQC + sucrose reached a maximum in fresh weight on the 4th day, then gradually decreased in weight and continued to elongate for five days. He further conducted another research and opined that gladiolus spike cv. White Friendship held in 600 ppm 8-HQC + 4 per cent sucrose sustained greater fresh weight, had more open florets and longer vase-life than gladiolus held in water.

Choi and Roh (1980) observed maximum vase-life and quality of gladiolus cv. Firebrand and carnation cv. Coral and Yellow Smiling was achieved with a solution of 8-HQC at 200 ppm + 2-4 per cent sucrose.

Commercially mature gladiolus inflorescence cv. Cammondo was treated with sucrose solution of different concentration after picking. The vase-life, general appearance, fresh mass and volume of medium uptake of the inflorescence improved with sucrose treatment and a concentration of 30g sucrose was most effective. Also, sucrose uptake from the vase solution replenished intracellular respirable carbohydrates, allowing a sustained high respiration rate and a prolonged vase-life (Marwe *et al.*, 1986).

The treatment of gladiolus cut flower spikes of the cv. Dukat, Lavenesque and Lustige Wittwe with a preservative solution consisting of 8-HQC at 200 ppm + GA3 at 60 ppm + sucrose at 30g/l markedly improved bud opening, increased spike length, floret diameter and the longevity of whole inflorescence.

Nagarjuna and Gowda (1998) found that when tuberose bulbs cv. Single was soaked in GA_3 (all at 100, 1000 and 1500 ppm) for 24h before planting increased flower spike fresh weight with all concentration compared with control. All treatments produced an increase in water uptake of cut flower spikes but water loss after 7 days was greater in all treatments and significantly increased the vase-life. Also, tuberose cv. Double when treated with GA_3 at 200 ppm showed longest vase-life, 7.67 days (Reddy *et al.*, 1997). However, cut gladiolus spikes cv. Oscar held in 5 per cent sucrose + 600 ppm 8-HQC sustained longest vase-life, 13.44 days (Suneetha Kumar, 1998).

De *et al.* (1998) elucidated that cut gladiolus cv. High Style spikes held in vase solution containing sucrose (4) per cent + 8-HQC (250 ppm)

improved the post harvest life (14.50days) and quality of cut gladiolus spikes.

Alvarez *et al.* (1994) observed that cut tuberose cv. Chapeada held in sucrose (3 per cent) + 8-HQC (200 ppm) showed greatest fresh weight throughout the experimentation.

Ichimura (1998) elucidated that sugar plays important roles in the keeping quality of cut flowers because the amount of sugar contained in cut flowers is limited and continuous treatment with sucrose (100g/l) markedly promoted floret opening and extended vase-life of cut Sweet pea (*Lathyrus odoratus*) flowers and also observed 50g/l sucrose was effective in improving vase-life cut flowers of snapdragon (*Antirrhinum majus*).

Lukaszewska (1995) stated that carnation cv. New Diana held in sucrose (5 per cent) + 8-HQC (200 ppm) increased the number of days until the last floret wilted and until the second floret wilted and also increased the number of open florets and vase-life. Whereas, chrysanthemum cv Polaris held in 8-HQC (200 ppm) + citric acid (75 ppm) + sucrose (5per cent) gave greater vase-life (Arriaga and Guerrero, 1995).

Zhang *et al.* (1995) revealed that buds of cut hippeastrum flowers kept in preservative solution increased flower diameter and enhanced colour and suggested that addition of GA_3 to the preservative solution improved the vase-life. Also, application of GA_3 in the preservative solution delayed floret senescence and increased petal size of cut *Strelitzea reginae*. It was found that sucrose is the exogenous carbohydrate source necessary for growth and development. Although it reduced the uptake of vase water and observed the reduction in the fresh weight of cut flowers began mainly 12 days after treatments.

Hwang and Kim (1995) stated the plant treated with GA_3 10 ppm or kinetin at 20 ppm, vase-life of cut flowers of *Gladiolus gandavensis* cv. Spic and Span extended. Flower quality was improved in terms of flower diameter, percentage of flowering, fresh weight, water uptake and anthocyanin content.

Pisulewski *et al.* (1989) reported that tulip cv. Christmas Surprise, Red Matador and Polka held in solution with 8-HQC (100 ppm) + sucrose (20g/l) responded positively to the treatment and addition of GA_3 (20ppm) to

the preservative solution significantly prolonged the vase-life and caused excessive stem elongation.

Goszczynska *et al.* (1989) observed sucrose (2 per cent) + 8-HQC (200ppm) as a preservative solution improved the keeping quality of cut daffodil flowers cv. Carlton.

2.6 EFFECT OF GROWTH REGULATORS ON BIO-CHEMICAL CHRACTERSDURING VASE-LIFE OF *gladiolus grandiflorus* L. cv. Jessica.

2.6.1 Enzymes:

A few numbers of reports are available regarding the increase and/ or decrease in activities of several hydrolyzing enzymes during vase-life and senescence of flower petals.

Peroxidase exhibits irregular drift in its activity during development and subsequent senescence. There is a considerable loss of protein in senescing leaves, whereas, peroxidase is not subjected to turnover (Axelrod *et al.*, 1951; Kar and Mishra, 1976 and Lewington *et al.*, 1967).

Parish (1968) suggested that the increase in peroxidase activity in sensceing tobacco leaf discs was associated with protein synthesis. He furthur suggested that the increase in peroxidase activity could be taken as a reliable indicator of leaf senescence.

The enzymic changes found during petal senescence are associated mainly with two major metabolic events: increase in respiration and hydrolysis of cell components. Increase in peroxidase was found in petals of Tobacco (Brendemeijer, 1973), Arundina (Lim *et al.*,1975), Phalaenopsis (Trippi and Tran Thanhvan,1971) and Tulip (Carfantan and Daussant,1975). The increase activity of peroxidase is apparently related to an increase in peroxides and free radicals that react with cellular constituents (Fridovich, 1975) and are involved in promotion of senescence (Baker *et al.*, 1977).

Tan and Hew (1973) observed the decreased polyphenoloxidase (PPO) activity in senescing corolla of Arachnis orchid.

Brennan and Frenkel (1977) opined the plausible possibility of senescence in flowers is that the compounded results leads to a higher oxidation state of the tissues, which may be in the form of accumulation of peroxides.

Paulin and Droillard (1989) investigated the membrane lipids peroxidation during the senescence of cut carnation (*Dianthus caryophyllus*) and observed the amount of peroxides increased from opening: when the petals form a 45° angle with the stem to withering and when the petals are wrinkled and discoloured and there is complete loss of turgor and decreased at total withering, also when the petals had collapsed and was shapeless.

Yamane *et al.* (1999) observed perianth peroxidase activity of gladiolus cv. Fuzinoyuki sharply increased within one day after their full unfolding, this increase was almost completely suppressed by addition of bactericides and they concluded that free radicals are involved with the increase in peroxidase activity and in the wilting process of gladiolus perianth.

Jordi *et al.* (1996) stated that increase in enzymatic activities the overall content of protein, RNA strongly decreased during leaf senescence of alstroemeria cut flowering stems and found that leaf senescence can be inhibited by GA_3 application and can also delayed, since the amount of protein increased much less (Van Doorn and Van Leiberg, 1993). However, Celikel and Doorn (1995) opined lipid peroxidation remain unaltered during the senescence of iris tepals.

Behera *et al.* (1990) reported that kinetin delayed petal senescence of cut carnation flowers and change in protein and enzyme activities (peroxidase) on leafy shoots.

2.7.2 Protein:

Celikel and Doorn (1995) reported that net loss of proteins in the tepal edges started after flower opening and after two more days, when the first symptoms of senescence were observed. The protein level was only 20 per cent of that at harvest during the senescence of iris tepals cv. Blue Magic.

However, in day lily tepals, a sharp decrease in protein levels preceded the visible symptoms of senescence (Lay- Yee *et al.*, 1992).

Sabehat and Zieslin (1994) stated membrane protein content suppressed when flower buds of rose cvs. Mercedes and Sonata were sprayed with 350-ppm solution of GA_3. The decrease in content of total protein in petals of GA_3 treated flowers was lower than that of control flowers and concluded that the post harvest decomposition of proteins was inhibited by the GA_3 treatment. Since, protein disintegration and decrease in protein content are symptoms accompanying post harvest senescence of flower petals (Halevy and Mayak, 1979; Borochov and Woodson, 1989).

Beja-Tal and Borochov (1994) studied the senescence related changes in flower petals of carnation and observed the contents of protein were lower in membranes isolated from old petals than in those from young petal.

Donald (1997) observed increased protein content in tulip tepals and then decreased during flower development, while protein content decreased in alstroemeria petals during senescence. Whereas, decreased protein concentration during petal development has been commonly observed by Matile and Winkenback, 1971 and increased protein concentration during petal development have been reported by Stead and Moore, 1977).

Weinstein (1957) stated that the reduction in protein level in rose petals cv. Better Times during senescence is initiated by the depletion of the carbohydrate reserves and exogenous application of sucrose will prevent the reduction in protein level.

Borochov *et al.* (1976c) observed during the course of petal aging there is a drop in the level of protein. Baumgartner *et al.*, 1975 and Weimken and Weimken, 1975 observed similar findings.

Parups (1971) observed less protein content in the senescing tissues of roses, carnation, chrysanthemum, snapdragon and kinetin treatments tended to maintain old leaf proteins comparable to fresh leaf proteins and suggested that proteins are significant in the processes of senescence and chemical control of proteins *in vivo* might help to maintain freshness in flower.

Racusen and Aranoff (1954) stated that decrease in protein content of excised leaves is not necessarily due to lack of carbohydrate or to an inability of the cell themselves to synthesize amino acids but is due to a failing ability to incorporate these amino acids into protein.

Richmond and Lang (1957) showed the protein loss could be retarded if excised leaves of xanthium are kept with their petiole dipping into solution of kinetin at 5 ppm. However, sprayed solution of kinetin directly on to leaves of Nicotiana may also retard leaf senescence by causing the treated areas to act as loci for the accumulation of metabolites viz. both protein synthesis and RNA synthesis is stimulated in kinetin treated parts of tobacco leaves. Thus, Moths and Engelbrecht (1961) conclude that 'accumulation ... is not the consequence of synthesis, but mass synthesis of protein for example is the consequence of an accumulation of amino acids'.

Chibnall(1954) found a rapid decrease in protein synthesis ultimately resulting in the death of detached runner bean leaves. However, the effect of kinetin at 60 ppm in the vase solution had no effect on the rate of reduction in protein content of cut rose flowers cv. Golden Wave (Mayak and Halevy, 1974).

Jiang *et al.* (1997) revealed decline in protein synthesis and the responsiveness to ethylene increased as the flowers of carnation cv. White Sim matured and concluded that changes in responsiveness to ethylene in flowers may be due to a decrease in the capacity for protein synthesis. While, soluble protein content increased initially and later decreased during vase-life of *Freesia refracta* cv.Auroia was observed by Su and Ye,1997.

2.7.3 Nucleic Acid:

Matile and Winkenbach (1971) reported that during the course of petal aging there is a drop in the level of nucleic acid. RNA content starts to decrease in morning glory even before anthesis while the sharp increase in RNase is evident only after the beginning of fading and this indicates the first stage in decline of RNA is caused by reduced synthesis, whereas degradation of DNA starts only after the onset of wilting, indicating autolysis

which signifies the death of cells. Similar finding were also observed by Stead and Moore, 1977).

Soleimani (1968) studied the effect of GA_3 on concentration of DNA, RNA and protein in pea plant (*Pisum sativum*) and concluded that the effect of GA_3 in causing stem elongation is associated with marked increase in DNA, RNA and protein content of tissues per unit of fresh weight. Protein and RNA showed relatively larger increase as compared to the increased amount of DNA and suggested that GA_3 is involved in stimulating the fundamental synthetic processes which cause the plant tissue to be more active in production of DNA and hence of RNA and protein.

Osborne (1962) concluded that a decline in levels of protein, RNA and DNA mark the progress of senescence in detached xanthium leaves but addition of kinetin at 40 ppm to detached leaves temporarily arrests the senescent changes and maintains a relatively high ratio of RNA (or protein) to DNA. The kinetin effect appeared to operate directly and is not dependent upon the accumulation of metabolites and suggested that the effect of kinetin in retarding senescence in xanthium leaf cells is mediated through its action in sustaining nucleic acid and protein synthesis.

Fletcher and Osborne (1965) studied the regulation of protein and nucleic acid synthesis by GA_3 during leaf senescence of *Taraxacum officinale* and observed in the leaf disc receiving GA_3, the synthesis of protein was not only maintained but also enhanced and the synthesis of RNA was also stimulated by the addition of GA_3. The specific activity of RNA was significantly enhanced by pre-treatment with GA_3 for only 1.5hours and concluded that the retardation of leaf senescence by GA_3 in *Taraxacum officinale* is closely linked to a regulation of protein and RNA synthesis and support the contention that hormonal retardation of leaf senescence is associated with maintenance of the DNA as a functional template for DNA dependent synthesis of RNA.

2.7.4 Carbohydrate:

The final stage of flower development is characterized by a decline in the content of carbohydrates (Mayak and Halevy, 1974). Generally, the senescence and wilting of the petals determine the longevity of the flower. The addition of sucrose in the vase-solution along with germicide extends the longevity of cut flowers (Aarts, 1957; Coorts, 1973). It also helps to slow the degradation of protein, release of ammonia and the consequent rise in the pH (Paulin, 1971) and result in flower size enlargement and the improvement of its colour (Parups and Chan,1973).

Marousky (1969a) observed cut mum flower buds held in 8-HQC + sucrose increased carbohydrate content in petals.

Nichols (1979) showed that the inclusion of sucrose in the vase solution of carnation flowers increased the concentration of glucose and fructose in the petals, as compared to control.

Bieleski and Reid (1992) studied the changes in sugar content of day lily petals during flower development and observed soluble carbohydrate was 50 per cent of petal dry weight up to 10hours, then decreased during senescence to reach 4 per cent by hour 34.

Moelem-Beno *et al.* (1997) observed GA3 enhanced sucrose uptake by detached corolla of petunia flower and noticed 20-30 per cent higher sucrose uptake than the control after incubation.

Donald (1997) found decreased carbohydrate concentration in tulip tepals during flower development. However, increased carbohydrate (sucrose) level in ray florets of *Zinnia elegans* if held in 200 ppm 8-HQC+ 3 per cent sucrose (Dennis *et al.*, 1983).

2.7.5 Sugars:

Flowers required imported carbohydrate for their development. The flower bud is a major sink for assimilates under favourable growth condition, whereas, a shortage of carbohydrate often leads to the arrest of flower development (Halevy, 1987). The role of sugars in flower development is

multifunctional: they can act as energy sources, as osmotic regulators and as precursors for metabolic processes (Kuiper *et al.*, 1991). Reducing sugar rather than sucrose were noted as the main constituents of sugar pool of mature petals (Kaltaler and Steponkus, 1974).

Paulin (1980) reported the accumulation of reducing sugars in the petals of roses fed with sucrose in vase solution.

Weiss *et al.* (1995) revealed that sugars play a central role in the regulation of petunia (*petunia hybrida*) flower development, which requires both sucrose and GA3 and also observed normal development and pigmentation of petunia flower can proceed only when sucrose supplied.

Donald (1997) observed high concentration of sugar in young tepals of tulip that declined as the tepals expanded and senesced.

Dennis *et al.* (1983) stated increased level of sugar (glucose and fructose) in ray florets of *Zinnia elegans* if held in 200 ppm 8-HQC + 3 percent sucrose.

Nichols (1976b) observed that an increase in the ratio of sucrose to reducing sugar is found in senescing carnation and ethylene triggered premature wilting.

An exogenous application of sucrose, increased the amount of reducing sugars appeared in the stem and then translocated to accumulate in the flowers, improving their ability to absorb water and maintain their turgidity (Halevy, 1976; Halevy and Mayak, 1974b).

Acock and Nichols (1979) suggested that sugars ability to delay senescence of cut carnation flowers is related to its ability to support cell metabolism and to maintain membrane integrity.

Nichols (1973) confirmed that absorbed sucrose impregnated in the vase solution is rapidly converted in petals to reducing sugars that accumulate in the corolla and help in delay of senescence.

Weinstein (1957) found glucose rather than sucrose to be the major constituent of rose petal tissues. Since the mechanism for glucose synthesis had in all probability been disrupted by detaching the rose bloom from the plant and by the subsequent handling treatment, a translocation of glucose from receptacle to petal tissues during the early stages of senescence seemed likely. He also found that proteolysis proceeded early and at a fairly

constant rate in the senescence process in cut roses and that it was independent of the level of a readily available respiratory substrate such as glucose.

Ichimura *et al.* (1999) investigated the physiological role of soluble carbohydrate in the petals of sweet pea and observed glucose; fructose and sucrose concentration increased during flower bud development and concluded that since the fresh weight of petal increased, the carbohydrate contents of petals increased.

Cut rose buds of cv. Super Star were placed in vase solution containing 8-HQC (250 ppm) + Acetylsalicylic acid (100 ppm) + D-fructose (1 per cent), contents of total soluble sugar and reducing sugar in corolla tissues increased sharply from harvest to third day in vases, thereafter, reduced at senescence of flower (Annonimous, 1996-97; Bhattacharjee, 1997a).

Roh (1990) investigated possible mechanism involved in bud abscission in the Asiatic hybrid lily (*Lilium elegans*) cv. Red Carpet and Sunray and observed sucrose content decreased from 2.91 to 0.64 mg/g fresh weight in Red Carpet and from 3.42 to 0.43mg/g fresh weight in Sunray. However, fructose content decreased and glucose content increased after abscission.

Kapchina (1989) estimated the content of sucrose and reducing sugars in the buds of spray carnation cv. Super Gold and showed the tendency of changing of the ratio of sucrose to reducing sugar was similar from the second till the 6^{th} days. At the stage of maximal withering the quantity of reducing sugars increased, as its level in the solution was higher during the period of bud development in comparison with the control.

Yamane *et al.* (1991) stated that during anthesis of cut gladiolus flower, fructose and glucose contents of the perianth increased up to complete anthesis and decreased at the wilting stage and was suggested that these soluble sugars are translocated from the perianth to other organs at the wilting stage.

2.7.6 Starch:

Donald (1997) observed decreased starch concentration in alstroemeria petals during its development and also found the starch concentration of tulip tepals was relatively low and gradually declined during development and stated starch, as dominant carbohydrate in alstroemeria petals throughout the growth period and a quantitative disappearance with a corresponding increase in glucose, fructose and to lesser extent, sucrose.

Ho and Nichols (1977) elucidated during the course of petal aging there is a drop in the level of starch. However, young petals of gladiolus contain high amounts of starch (Ramanuja Rao and Mohan Ram, 1979).

Commercially mature gladiolus inflorescence cv. Commando were treated with sucrose solution of different concentration and was found that when 30g sucrose/dm^3 added to the vase solution, the activities of α and β-amylase responsible for the noticeable decline in the starch concentration immediately after harvest, were lower during the final stages of senescence (Marwe *et al.*, 1986).

Yamane *et al.* (1991) stated that during anthesis of cut gladiolus flower, starch content was high initially then decreased during development indicating that starch is the primary source of soluble carbohydrate during the early stage of flower expansion.

2.7.7 Phenol:

In some flowers aging of petals is marked by browning and blackening of the petals, which are caused by oxidation of flavones, leucoanthocyanins and other phenols and the accumulation of tannins (Singleton, 1972).

The final stages of senescence results from the leakage of the substances such as phenols from vacuole and their consequent interaction with the remaining functional elements of the cell as reported by Liebermann and Biale, 1956; Mayer and Friend, 1960.

Weinstein (1957) reported approximate 90mg of tannins per bud of Better Times rose and found the total quantity of tannins in the petals decreased slightly during senescence.

Durkin (1967) observed that injured cells at the cut ends of rose released tannins and the enzyme peroxidase results senescence of the cut rose flowers.

2.7.8 Pigments:

Colour fading and discoloration is an important factor in determining display quality of cut flowers and in many cases is the major reason for the termination of vase-life. In spite of this, most studies on post harvest handling of cut flowers do not present data on the changes in pigmentation and those that do so use subjective colour grades for evaluation. Only in a few studies have been used for objective definition of the colour changes in cut flowers. The major types of pigments contributing to the colour of the flowers are carotenoides and anthocyanin.

Celikel and Doorn (1995) showed an increase in anthocyanin content, prior to the visible senescence symptoms of iris tepals during vase-life.

Moalem-Beno *et al.* (1997) observed when GA_3 and sucrose were supplied together to detached corolla of petunia flower, a high level of anthocyanin, about 4 times that found within other treatments.

Justisen *et al.* (1997) studied the accumulation of anthocyanin during bud and flower development in *Campanulla isophylla* and showed anthocyanin content were very low in buds until a few days before anthesis, after which they increased rapidly until about 4 day after anthesis, then a decline became established. The decline affected both anthocyanin concentration and anthocyanin content per flower. However, rapid accumulation of anthocyanin in petals has commonly been observed in the later stages of flower development (Davies *et al.*, 1993), and it has been shown that this rapid increase in anthocyanin is caused by de novo synthesis viz. in *Hibiscus mutabilis*(Amerheim and Frank,1989), degradation of anthocyanin as flowers age has been observed in chrysanthemummum

(Stickland,1972), in contrast to *Mathiola incana* whose anthocyanin are quite stable(Dangelmayer *et al.*,1983).

Zieslin and Halevy (1969) reported that GA_3 treatment caused accumulation of anthocyanin in petals of Baccara roses, whereas promotion of anthocyanin biosynthesis in rose petals by cytokinin was studied by Nakamura *et al.*, 1974).

Only a few studies have been carried out on the changes in carotenoids in aging flowers. A decrease in total carotenoid content was observed in senescing chrysanthemum flowers (Stickland, 1972). While an increase in oxygenated carotenoides with age was found in *Strelitzia reginae* (Simpson *et al.*1975) and rose (Valadon and Mummery, 1969) flowers and was considered as a sign of degenerative and uncontrolled oxidative process (Goodwin, 1966).

Much more is known about the change in pigmentation due to anthocyanin. There is no universal trend in the anthocyanin content in aging petals. The pigment level stays stable in some flowers and declines drastically in others, while in some flowers a dramatic synthesis of anthocyanin is evident. An increase in anthocyanin formation with wilting is one of the typical post pollination phenomena in *cymbidium* orchids (Arditi and Flick, 1976; Arditi and Knauft, 1969).

Nielson and Bloor (1997) described the changes in pigment content during floral development from a bud to a fully mature open flower of petunia and showed in cv. California Girl carotenoid level steadily increased as the flower matured. The cv. Summer Sun was characterized by a significant increase in α-carotene levels in flower greater than 5cm in length, petals fully loosened and pigmentation becoming evident, also α-carotene levels in Bright Yellow were maintained in the immature petal tissues. They also concluded that carotenoid levels increased during development and much higher levels were present in dipper than in lighter colored petal tissues indicating that carotenoids contribute the deeper petal colour and overall impression of yellow.

Merzlyak *et al.* (1999) used the application of non-destructive reflectance spectroscopy for assessment of the physiological state of the

plants and monitored senescence induced events. They observed remarkable difference between reflectance spectra of coleus related to the difference in pigment transformation during senescence in coleus, degradation of carotenoid occurred. Simultaneously only trace amount of carotenoid were present in senescent leaves.

In trials with the gladiolus cv. Eurovision, cormels were soaked for 24hours in solution of GA_3 at 0-500 ppm and was found that flower colour was deeper in the treated plants due to higher anthocyanin content (El-Meligy, 1982).

Suneetha and Kumar (1998) observed that cut gladiolus spikes of cv. Oscar held in 5 per cent sucrose + 600 ppm 8-HQC showed greatest anthocyanin pigment retardation.

Ichimura (1998) revealed continuous treatment with sucrose (20g/l) + 8-HQC (200 ppm) increased the anthocyanin concentration in petals and extended the vase-life of cut flower of *Eustoma grandiflorum*. However, maximum anthocyanin content was first attained about 5 days after flowers appeared *Campanula isophylla*, thereafter, a slow decline occurred (Hansen and Justesen, 1996).

Kazakidon and Burrage (1994) stated that flower carotenoid content (mg/g DW) increased to a maximum in 3 weeks after the first flower reached the half open stage then remained constant throughout the harvest period.

2.7.9 pH:

Reist (1977) studied the composition of pH of a nutrient solution on flower quality and vase-life of gladiolus cv. Oscar and indicated that gladiolus spike produced flowers nicely in nutrient solution containing 200 ppm 8-HQC and 35g/l sucrose, lowering the pH 3.5 with citric acid. Whereas, Jones and Meagan (1993) revealed the pH of the HQC solution did not appear to affect fresh weight or vase-life of rose, gerbera, gypsophyla, carnation and chrysanthemum.

A decrease of vase solution pH to well below 7 clearly promotes flowers water uptake (Durkin, 1979b and Conrado *et al.*, 1980).

Durkin (1979a) observed low pH (3-3.5) increased the rate of flow in isolated 5cm stem segments of rose flowers and elucidated acidification of the water greatly improve water uptake (Durkin,1981).

Pokorny and Kamp (1953) reported little or no effect on the keeping quality of carnation, rose and stocks with the use of preservative materials alone, but that a marked increase in the keeping quality resulted when the aqueous solution was acidified to about pH 4 prior to adding the preservative. They stated that pH level was not critical, but that it should be within the range of 3 to 5.0. This relationship between pH level and shelf life was disputed by Stoltz (1956) who felt '...any increase in shelf life attributed to acidification was either non-existent or of small magnitude'.

Marousky (1971c)observed low pH (3.0 to.4.0) of flower preservative solution results in improved keeping life. Whereas, holding roses in a preservative solution containing 8-HQC and sucrose prevented the increase in pH and the resultant colour change (Aarts *et al.*, 1957).

Phavaphutanan and Ketsa (1989) reported that vase-life of cut roses cv. Christian Dior tended to increase as the pH was lowered and concluded that lowering the pH increased the fresh weight, water conductivity and water consumption of cut roses.

2.8 Respiration and Ethylene production:

The rate of respiration in many flowers rises to a maximum as flowers start to open, followed by a gradual decline as flowers mature. Then, it increases dramatically over a relatively short period and finally decline. The second peak in the respiration drift is considered to indicate the final senescence stage.

Water stress in plants may lead to increased ethylene production (Apelbaum and Yang, 1981). A rise in ethylene production during flower development has been detected in cut gladiolus spike. Ethylene may inhibit or stimulate flower opening in cut gladiolus. Some flowers in which petals wilt when they senesce show regulation of senescence by ethylene, whereas in others petal wilting is apparently not regulated by ethylene (Woltering and Van Doorn, 1988). In species where petal regulation is ethylene-regulated

water stress may thus lead to advanced senescence viz. carnation, gladiolus, day lily, tulip etc.

Roh (1990) investigated possible mechnism involved in bud abscission in the Asiatic hybrid lily (*Lilium elegans*) cv. Red Carpet and Sunray, respiration rate decreased from 480 to 200mgCO_2/kg/h in Red Carpet and from 300 to 200mgCO_2/kg/h in Sunray as bud length increased from 1.8 to 6.5 cm. A peak in ethylene production occurred concomitantly with the CO_2 peak.

Celikel and Doorn (1995) revealed the rate of respiration of iris tepal remains unchanged and their rate of ethylene production decreased during senescence. However, the ethylene production of gladiolus cv. Spic and Span flower spikes after harvest showed a typical climacteric pattern and suggested ethylene is involved in the senescence of gladiolus flower and also observed that the treated flowers with GA_3 at 10 ppm or kinetin at 20 ppm exhibited reduced ethylene production and reduced respiration rate (Hwang *et al.*, 1995).

Donald (1997) observed rapid increase in respiration in both tepals and petals of tulip and alstroemeria prior to flower opening, had decreased 23 and 30 per cent, respectively by the time the flower had fully opened and during senescence.

Dennis *et al.* (1983) elucidated ethylene biosynthesis was enhanced by holding flowers of *Zinnia elegans* in solution of 8-HQC + 1, 2, 3 per cent sucrose compared with deionized water, where ethylene release was low initially and remained low. Carbon dioxide evolution declined sharply the first two days post harvest and remained low for flowers held in deionized water, but remained at initial levels for those held in 200 ppm 8-HQC + 3 per cent sucrose.

Siegelman (1952) reported an initial high level of respiration and gradual decrease with time for both the Better Times rose and Mystery gardenia and observed the respiratory sequence was attributed to an accumulation of starch and its subsequent disappearance.

Siegelman *et al.* (1958) describe a definite respiratory pattern in developing rose petals, which was dependent upon the location of the petal of the flower. If the five outer petals were used, the pattern consisted of the

respiratory rise before the opening of the flower, a peak when the flower fully opened and a drop following the complete opening of the flower. The increase in respiration during the climacteric stage of the flower was accompanied by an increase in fresh weight and cell size. However, respiratory rates of carnation flowers were 3 to 4 fold greater than chrysanthemum and concluded that a direct relationship appear to exist between respiration rate and longevity(Kuc and Workman,1964).

Coorts *et al.* (1965) observed the respiratory pattern of Velvet Times rose cut at stages prior to time of commercial harvest as compared to post harvest respiration. They found the respiratory rate for flowers cut pre-harvest was quite high and reached the maximum at the time when the sepals had folded out from the developing bud. At commercial harvest, when the first petals were breaking away from the flower body, respiration was declining rapidly. The minimum post harvest respiratory rate was attained on the 3rd day after harvest.

Marousky (1969) reported that sucrose in the vase-solution increased the respiratory rate of Better Times rose petals but 8-HQC did not influence respiration, whereas combined effect of 8-HQC with sucrose delayed senescence of Red Sim cut carnation, promoted water flow through basal stem sections and delayed the climacteric peak.

2.9 EFFECT OF GROWTH REGULATORS ON CORMS CHARACTER OF *Gladiolus grandiflorus* L cv. Jessica.

Arora *et al.* (1992) observed that gladiolus cv. Aldebaran, Pusa Suhagin and Mayur exhibited differential response to GA_3 treatment in terms of size and weight of cormels and showed maximum effect in cv. Mayur where, 100 ppm GA_3, caused 239.39 and 59.13 per cent increase in cormel weight and diameter, respectively, over the untreated ones. Production of cormlets did not change significantly by GA_3 application but there was an appreciable increase in their weight and size accompanied with cv. Mayur.

Leena *et al.* (1992) showed significant effect of growth regulators on the weight and size of corms and yield of cormels and observed heavy

corms weighing 55.61g with GA_3 50 ppm, maximum number of cormels with GA_3 100 ppm 91.33 and maximum weight of cormels with GA_3 50 or 100 ppm, of cv. Freindship.

Mukhopadhyay and Bankar(1986) noticed a reduction in the number of cormels produced per plant when corms of gladiolus cv. Freindship were soaked with 0, 10, 50, 100, 250, 500 ppm GA_3 in the dark for 24h, as compared to those from control plan. However, Winkler (1969) reported increase in corm yield and cormel weight only by dipping with GA_3 in contrast to spraying. Increase in cormel yields following GA_3 application had also been reported by other workers (Bhattachargee, 1984 and Dua *et al.*, 1984).

Bhattachargee (1984) studied the effect of soil drench application of GA_3 (10, 100 or 1000 ppm each) in cv. Friendship and reported that GA_3 at 10 or 100 ppm improved corm size and weight and induced more cormel production.

Mukhopadhyay and Banker (1983) revealed that plants of the tuberose cv. Single sprayed at 40 days after planting and twice more at fortnightly intervals with GA_3 at 25-100 ppm inhibited rhizome production at all concentrations. However, gladiolus cv. Tropic Sea corms soaked for 24hours in aqueous solution of GA_3 at 40 ppm gave greatest number (3.5) of cormels per plant (Pal and Chowdhary, 1998).

Preeti *et al.* (1997) elucidated that pre-planting treatment of bulbs of tuberose cv. Single with GA_3 (50, 100 or 200 ppm) reduced the number of bulbs produced per plant irrespective of the treatments. Whereas, application of kinetin at 500 or 1000 ppm to the Lilium Asiatic hybrid cv. Toejon 52 promoted the bulblet weight (Suh *et al.*,1995).

MATERIALS AND METHODS...

The present field investigation entitled **'Studies on the effect of VAM and growth regulators on *Gladiolus grandiflorus* L. cv. Jessica'** was carried out at the experimental area and laboratory of the department of Horticulture, CCS Haryana Agricultural University, Hisar, India during 1999-2000 and 2000-2001, respectively. However, bio-chemical studies were carried out only during first year of investigation. Jessica cultivar of gladiolus, being one of the most popular and important, was selected for experimentation. The cultivar provides excellent pink colour flower spikes, which are used as a cut flower. The details of material used and procedure followed during the course of experimentation are mentioned here under.

3.1 Selection of cultivar:

The present investigation was carried out on cultivar Jessica, comes under plurifoliati group, having 5-8 or more well developed leaves, distichously arranged, forming a fan. *Gladiolus grandiflorus* cv. Jessica is a large or exhibition type grows to a height of 90-150cm with strong erect stalks, bearing closely arranged triangular and symmetrical flowers with pink colour florets between 12-20. Jessica is a mid season culltivar of spike length 90-95cm, a hardy and good cultivar. Corms 1.5-2.5cm in diameter were planted on the experimental field.

3.2 Nutrilink (VAM fungi):

Vesicular arbuscular mycorrhiza (V.A.M) was obtained from division of microbilogy, I.A.R.I, New Delhi. VAM fungi, inoculant virtually form association with all the land plants, field crops and trees (orchard, forest) and can be used to improve the establishment and further plant health of agricultural crops, vegetable, horticultural, floricultural and forestry tree plant. VAM fungi increase the plant growth and health by improving the availability of soil phosphorus and enhance the uptake of applied fertilizer, nitrogen.

Nutrilink (VAM bio-fertilizer) is eco-friendly and it brings good effect on plant growth even if high quantity is applied.

3.3 Preparation of experimental field:

The experimental field was prepared well by repeated ploughing followed by rolling for a fine filth. Required area was marked and plots were laid out according to the plan of lay out. Corms of the cultivar Jessica were planted in mid October at the depth of 10cm. Recommended doses of F.Y.M 55 tons/ha, 115 kg/ha N_2, 135 kg/ha P_2O_5, and 114 kg/ha K_2O were applied to the crop. Full dose of phosphorus and potash and 1/3 rd of nitrogen were applied as basal dose at the time of planting of gladiolus corm cv. Jessica. Rest of the nitrogen was applied in two equal doses as top dressing, one at the time of spike emergence and the second dose after harvesting the spikes for providing nourishment to the growing corms. Irrigation to the experimental field was given from time to time as per the recommendation and prevailing weather conditions.

3.4 Details of planting:

Date of planting	:	20th of October (1999 and 2000)
Spacing	:	30 x 30cm
Number of rows in the plot	:	4
Number of corms in the row	:	4
Total corms in each plot	:	16

3.5 Details of lay out of the experiment:

Design	:	R.B.D (Field experiment)
		C.R.D (Lab experiment)
Number of replications	:	3 (Field experiment)
		4 (Lab experiment)
Number of treatments	:	13 (Each for experiment 1 and 2)
Total number of plots	:	13 x 3 = 39
Net size of the plots	:	1.2 x 1.2m
Main irrigation channel	:	1m

Sub irrigation channel	:	0.5m
Field border	:	1m
Plot border	:	0.20m
Field size	:	22.20 x 11.90m

3.6 Details of treatments:

The experiment was conducted in RBD and CRD, with replication three and four. The treatments were allocated randomly in each plot using Fisher and Yates allocation random table (Panse and Sukhatme. 1995). The symbols assigned to different treatments are given as under for experiment 1 and 2.

Experiment 1. Studies on the effect of VAM and growth regulators on *Gladiolus grandiflorus* L. cv. Jessica.

Symbols		Treatments
T_1	:	Control
T_2	:	Pre- soaking in GA_3 100ppm +VAM
T_3	:	Pre- soaking in GA_3 200ppm +VAM
T_4	:	Pre- soaking in Kinetin 50ppm +VAM
T_5	:	Pre- soaking in Kinetin 100ppm +VAM
T_6	:	Foliar spray GA_3 100ppm +VAM
T_7	:	Foliar spray GA_3 200ppm +VAM
T_8	:	Foliar spray Kinetin 50ppm +VAM
T_9	:	Foliar spray Kinetin 100ppm +VAM
T_{10}	:	Pre- soaking and Foliar spray GA_3 100ppm+VAM
T_{11}	:	Pre- soaking and Foliar spray GA_3 200ppm +VAM
T_{12}	:	Pre- soaking and Foliar spray Kinetin 50ppm +VAM
T_{13}	:	Pre-soaking and Foliar spray Kinetin100ppm +VAM

Experiment 2. Studies on the effect of growth regulators on *Gladiolus grandiflorus* L. cv. Jessica.

Symbols		Treatments
T_1	:	Control
T_2	:	Pre- soaking in GA_3 100ppm
T_3	:	Pre- soaking in GA_3 200ppm
T_4	:	Pre- soaking in Kinetin 50ppm
T_5	:	Pre- soaking in Kinetin 100ppm
T_6	:	Foliar spray GA_3 100ppm
T_7	:	Foliar spray GA_3 200ppm
T_8	:	Foliar spray Kinetin 50ppm
T_9	:	Foliar spray Kinetin 100ppm
T_{10}	:	Pre- soaking and Foliar spray GA_3 100ppm
T_{11}	:	Pre- soaking and Foliar spray GA_3 200ppm
T_{12}	:	Pre- soaking and Foliar spray Kinetin 50ppm
T_{13}	:	Pre- soaking and Foliar spray Kinetin100ppm

3.7 Application of growth regulators and (Nutrilink)V.A.M fungi:

Two plant growth regulators namely GA3 and kinetin were used during the experimental period. Corms were dipped for 24 hours separately in GA_3 100, 200ppm and kinetin 50, 100ppm, respectively a day before planting of gladiolus corms. Corms were dipped in respective plant growth regulators after removing the tunica (dry scales). Care was taken while using GA_3 solution, since it lost its effectiveness in the presence of light, hence corms were dipped under dark condition and left for 24 hours in dark. Foliar spray in the respective treatment combinations was applied at 45 days after planting the corms.

Nutrilink (VAM fungi) was applied at the time of planting of corms. The inoculant was mixed with soil on per plant basis i.e. 20 g/plant.

3.8 Observations:

Observation on different characters was recorded from time to time. Vegetative growth characters included number of days required for sprouting of corms, plant height, number of leaves per plant and leaf area while flowering characters included number of days required for spike initiation, total number of florets per spike, fresh weight of spike at the time of harvesting, physiological and bio-chemical changes during vase-life after keeping in standard vase solution and corm characters were also observed. The length of the plant was recorded with the help of a meter scale. The number of leaves at 30 and 60 days after planting as had emerged were also counted. The extent of flowering was determined by counting the number of florets per spike and measuring the length of rachis by the help of a meter scale at different stages of crop growth.

3.8.1 MORPHOLOGICAL AND VEGETATIVE CHARACTERS:

Number of days required that for sprouting of corms and after sprouting, different growth aspects were recorded from each treatment. The observations were recorded on 5 sample plants and then the average was worked out.

3.8.1.1 *Number of days required for sprouting of corms*:

Day to day observations was commenced for sprouting of corms and day was recorded on which corms were started to sprout.

3.8.1.2 *Percentage of sprouting at 30 days after planting*:

Total number of corms counted which were sprouted at 30 days after planting and was divided with total number of corms planted in each plot. Percentage was recorded by multiplying it with 100.

3.8.1.3 *Plant height (cm)*:

The height of the plant after sprouting was recorded at 30 and 60 days interval from the base and when the plant had attained a constant height.

3.8.1.4 *Number of leaves*:

On each selected shoot of the plant, number of leaves emerged were counted and recorded at 30 and 60 days after planting. The average number of leaves per plant was calculated.

3.8.1.5 *Leaf area (cm^2)*:

Leaf area was recorded at the time of spike harvesting with the help of leaf area meter.

3.8.2 FLOWERING PARAMETERS:

3.8.2.1 *Number of days required for spike initiation*:

Day to day observation was made to check the appearance of spike initiation and time taken for emergence was recorded.

3.8.2.2 *Total number of florets per spike*:

The number of florets was counted on the day when the last floret opened on the spike and average was calculated.

3.8.2.3 *Fresh weight of spike*:

The spikes were harvested from the 2^{nd} or 3^{rd} pairs of leaves at tight bud stage, then all the spikes were recut to equal length and weighed carefully on an electronic balance and average was calculated and at

several day intervals and data were recorded as increase in fresh weight in grams.

3.8.3 *Physiological parameters during vase-life*:

The spikes of gladiolus cutivar Jessica were cut at tight bud stage just showing colour of one or two florets from the 2nd or 3rd pairs of leaves and placed in water at the field, then these werebrought to laboratory and recut to a equal length and were kept at standard solution containing 4 per cent sucrose + 200ppm 8-HQC. The mouth of 500ml conical flask was tightened with cotton to avoid the transpiration from the open space. The pH of the standard solution to 3.5 was maintained by adding citric acid and then after physiological and bio-chemical changes during vase-life and the longevity of the spike was studied.

3.8.3.1 *Elongation of flower spike (Daily)*:

Spike length was measured from the base of the lowest floret to the spike apex and growth recorded as the increase in overall length expressed in mm.

3.8.3.2 *Length of flower (cm)*:

Length of flower was recorded one day before the anthesis of the second floret of each spike with the help of digital vernier calipers.

3.8.3.3 *Diameter of flower (cm)*:

The diameter of the second floret of each spike was measured by taking width of floret in two directions i.e. where it was maximum and minimum with the help of digital vernier calipers. The average of these values was considered as the diameter of floret.

3.8.3.4 *Water uptake and water loss by cut spike (ml/spike/day)*:

Cut gladiolus was fitted individually into cotton, which plugged a 500ml conical flask allowed Water loss from the system was only via the flower. At the start of the experiment and at an interval (daily) specified in the experiment, the weight of the flask including the flower (a) and without the flower (b) was recorded. This procedure was followed till the flower showed sign of wilting.

The differences between consecutive weighing of (b) were recorded as the amount of water absorbed and the differences between consecutive weighing of (a) were recorded as the quantity of water transpired.

3.8.3.5 *Flower quality*:

Several criteria were used to evaluate flower quality namely, time required for opening of basal floret, the number of days to senescence of basal floret, the number of florets per spike open at the time of senescence of basal floret and the number of florets per spike open before wilting or stem collapse through day to day observations.

3.8.3.6 *Longevity*:

Longevity was measured from the time the flower was cut to the termination of their vase-life. Flowers were considered to terminate their life when the whole flower wilted or when advanced sign of fading were visible in all petals (Halevy and Kofranek, 1977).

3.8.4 BIO-CHEMICAL ANALYSIS:

3.8.4.1 Enzymes:

3.8.4.1.1 *Peroxidase* (EC 1.11.17):

The peroxidase activity was assayed by the method of Summer and Gjessing (1943) as described by Kaul and Farooq (1994).

Extraction:

Four g of petals was homogenized in 8 ml of chilled 0.2M tris-HCL buffer (pH 7.5) containing 0.1M each of cystein and EDTA. The homogenate was centrifuged at 15,000 g.in a refrigerated centrifuge at a temperature of 4°C for 15 minutes. The supernatant was used for enzyme assay.

Reagents:

1. O-dianisidine 0.01 M in methanol (fresh).
2. H_2O_2 0.02 M (0.2ml H_2O_2 was dissolved in 100 ml distilled water (fresh).
3. Phosphate buffer 0.01 M (pH 6.0).

Assay:

The reaction mixture contained 1 ml of 0.01 M o-dianisidine in methanol, 0.5 ml of 0.02 M H_2O_2, 1ml of 0.01 M phosphate buffer (pH 6.0), 2.4 ml distilled water and 1 ml enzyme extract. The reaction was initiated by the addition of H_2O_2 and change in absorbance was noticed at 430 nm in spectronic-20. The enzyme activity was calculated as change in optical density of reaction mixture per second per mg of petal tissue.

3.8.4.1.2 *Polyphenol oxidase* (EC 1.10.3.1):

Polyphenol oxidase was assayed by the method given by Okagamy (1979) as described by Kaul and Farooq (1994).

Extraction:

The enzyme extract obtained for peroxidase was used for polyphenol oxidase.

Reagents:

1. Catechol solution 0.05 M: It was prepared by dissolving 0.55g of catechol D-dihydroxy benzene in 100ml of 0.03 M phosphate buffer (pH 6.0).
2. Tricholoro acetic acid (TCA) 10% w/v: 10g of TCA were dissolved in 100ml distilled water.

Assay:

1 ml of enzyme extract was incubated with 4ml of 0.05 M catechol solution at 30°C for 20 minutes. The reaction was terminated by adding 1ml of chilled 10% TCA. The optical density was recorded at 430 nm against the reagent blank. One unit of enzyme represents increase in optical density at 1.0 under the standard condition.

3.8.4.2 Protein content:

The proteins were estimated by the method of Lowry *et al.* (1951) using Folin-Cicalteau's reagent.

3.8.4.2.1 *Total proteins*:

Extraction

500mg of fresh petal tissue were homogenized with 5ml of 1N NaOH solution. It was kept for 24 hours for hydrolization and then centrifuged at 5000 rpm for 15 minutes. The supernatant was used for protein estimation.

Reagents

a. 2% Sodium Carbonate solution in 0.1 N NAOH.
b. Copper Sulphate solution (0.5%) in 1% Sodium Citrate solution.
c. 1ml of b + 50ml of a.
d. Folin-Cicalteau's reagent.

Estimation:

To a 0.1 ml of aliquot, 1 ml of reagent c was added and it was incubated for 10 minutes. After that 0.2 ml Folin-Ciocalteu's reagent was added and mixed thoroughly. After 15 minutes, the volume was made to 5 ml and optical density was recorded at 750 nm. A standard curve was prepared with graded concentrations of bovine serum albumin, and the volume was expressed as mg/g of dry weight.

3.8.4.2.2 ***Water soluble protein*:**

500mg of fresh petal were homogenized in 5 ml of distilled water. It was shaken vigorously for 5 minutes. After 15 minutes, it was centrifuged at 5000 rpm for 10 minutes. The supernatant was used for estimation of water-soluble proteins.

Reagents:

Same as for total proteins.

Estimation:

Same as for total proteins.

3.8.4.3 ***Nucleic acid (DNA and RNA)*:**

Diphenylamine and acid orcinol method did the quantitative estimation of DNA and RNA, respectively as suggested by Sadasivam *et al.* (1975).

Extraction:

Extraction procedure for the nucleic acid was adopted according to Smillie and Krotkov (1960) with slight modifications.

One gram of petals was homogenized with 10ml of ice cold 10 per cent tricholoro acetic acid (TCA) and the contents were centrifuged at 3000

rpm for 10 minutes. Supernatant was discarded and the residue was suspended in 5ml ice cold TCA. The contents were again centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded and the residue was suspended in 5ml ethanol-ether (3:1) mixture. The contents were centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded. To the residue 5ml of 0.5 per cent NaOH was added mixed well and left at 37^{o}C for 18 hours. The contents were again centrifuged at 3000 rpm for 10 minutes. The supernatant had both RNA and protein in hydrolized form. The residue contained some proteins and most of the DNA.

To the supernatant added 5ml of 10 per cent TCA. This was centrifuged at 3000 rpm for 10 minutes. The residue was discarded and supernatant contained nucleotides released from RNA. This was made to a known volume and RNA was estimated as per the procedure given below.

To the residue 1ml of perchloric acid was added and heated in boiling water for 1 hour and then cooled. This was again centrifuged at 3000 rpm for 10 minutes. The residue was discarded. The supernatant contained nucleotides released from DNA. This was made to a known volume and DNA was estimated as per the procedure given below.

Estimation of RNA:

The supernatant, which was contained nucleotide released from RNA, was made into a known volume.1ml of aliquot from this known volume was taken in the test tube and made to 3ml with distilled water. Then about 6ml of orcinol acid reagent was added to each test tube and then 0.4ml of alcoholic orcinol was added to each test tube and mixed thoroughly. The contents were heated in the boiling water bath for 20 minutes, a green colour was developed. Test tube was cooled and the absorbance read at 660 nm against the blank.

A standard curve was drawn using A_{660} the concentration of standard RNA on ordinate and abscissa side, respectively. The amount of isolated RNA solution was calculated (µg/g) using the graph.

Estimation of DNA:

The supernatant was made into a known volume of 5ml with distilled water. 1.5ml of the aliquot from this known volume was taken in the test tube and made to 3ml with distilled water. 6ml of diphenylamine reagent was added and mixed well. It was heated in boiling water bath for 10 minutes, a blue colour was appeared. The test tubes were cooled and the absorbance of blue solution was read at 600 nm against the blank.

A standard was constructed A_{600} versus quantity of DNA and then the concentration of DNA was calculated (μg/g).

3.8.4.4.1 ***Total carbohydrate:***

Total carbohydrate was estimated by the method of Hedge and Hofreiter(1962) using anthrone reagent.

Extraction:

500mg of petal was homogenized with 5ml of 2.5N HCL and hydrolyzed it in boiling water bath for three hours and cooled to room temperature and then neutralize it with solid sodium carbonate until effervescence ceases and total volume was made to 100ml and centrifuged. The supernatant was used for determination of total carbohydrate.

Reagents:

1. 2.5N HCL
2. Anthrone reagent (0.2 per cent): It was prepared fresh by dissolving 200mg of anthrone in 100ml of ice cold H_2SO_4.

Estimation:

0.1ml of aliquot was taken from the supernatant and was made volume to 1ml by adding distilled water. Then 4ml of anthrone reagent were

added to it and heated for 8 minutes in a boiling water bath, a green colour was developed. The absorbance was recorded at 620 nm against the reagent blank. The standard curve was prepared using graded concentration of glucose. The value was expressed in mg/g fresh weight.

3.8.4.4.2 ***Reducing sugar***:

Reducing sugar was estimated by the method of Miller (1972) using dinitrosalicylic acid reagent (DNS reagent).

Extraction:

100mg of petal were homogenized with 80 per cent ethanol twice (5ml each) and centrifuged it at 5000 rpm for 10 minutes. The supernatant was collected and evaporated by keeping it on a boiling water bath, After evaporation, 10ml distilled water was added and was used for determination of reducing sugar.

Reagents:

1. Dinitrosalicylic acid (DNS reagent): DNS reagent was prepared by *dissolving 1g dinitrosalicylic acid, 200mg crystalline phenol and* 50mg sodium sulphite in 100 ml 1 per cent NaOH by continuous stirring.
2. 40 per cent Rochelle salt solution (Potassium sodium tartarate).

Estimation:

1.5ml aliquot was taken from the solution and equalized the volume to 3ml with distilled water. Then 3ml of DNS reagent were added and content was heated in a boiling water bath for 5 minutes. When the contents were still warm, 1ml of 40 per cent Rochelle salt solution was added. The absorbance of red colour was recorded at 510 nm. The standard curve was prepared using graded concentration of glucose. The value was expressed in mg/g fresh.

3.8.4.4.3 *Non reducing sugar*:

Non reducing sugar was calculated by subtracting total carbohydrate with reducing sugar and multiplying it with 0.95.

3.8.4.5 *Starch*:

The starch was estimated by the method of Hassid and Neufeld (1964).

Extraction:

5ml of 26 per cent $HCLO_4$ were added to the residue, which was left after extracting the total carbohydrate. It was kept for 48 hours at 4°C in refrigerator. Then it was centrifuged at 5000 rpm for 10 minutes. The supernatant was used for starch estimation.

Reagent:

Anthrone reagents (0.2 per cent) as in total carbohydrate.

Estimation:

0.1ml aliquot was taken in a test tube. To it was added 0.9ml distilled water and it was mixed thoroughly. Then 4ml anthrone reagent was added and mixed well. It was kept on boiling water bath for 10 minutes and cooled. The absorbance was read at 620 nm against the reagent blank. The concentration of starch was calculated using the soluble carbohydrate standard curve and then it was multiplied by 0.9 (0.9g starch yield 1g of glucose). The values were expressed as mg/g fresh weight.

3.8.4.6 *Phenol content*:

The total phenol content were estimated by the method of Amorium *et al.*, (1977), using Folin-Ciocalteu's phenol reagent.

Extraction:

The extract obtained for total carbohydrate was also used for the estimation of total phenols.

Reagents:

1. Folin-Ciocalteu's phenol reagent (1N).
2. Sodium carbonate solution (20 per cent): It was prepared by dissolving 20g sodium carbonate in 100 ml distilled water.

Estimation:

0.5ml aliquot was taken from the extract and to it 4ml distilled water was added. To this 0.5ml Folin-Ciocalteu's reagent was added and mixed properly. After 5 minutes, 0.5ml of 20 per cent Na_2CO_3 solution was added. It was mixed vigorously and allowed to stand for one hour. The total volume was made to 10 ml with distilled water. The optical density was read at 750 nm against reagent blank. Standard curve was prepared by using graded concentrations of catechol. The values were expressed in mg/g fresh weight.

3.8.4.7 *Anthocyanin content*:

The anthocyanin content were estimated by the method of Fuleki and Francis(1968).

2g petal was homogenized with ice cold 10ml (5 + 5ml) 85:15 (95 per cent ethanol + 0.1N HCL) of pH 2.0 and centrifuged at 5000 rpm for 10 minutes. The supernatant was used for estimation of anthocyanin contents.

Estimation:

0.2ml aliquot was diluted to 5ml with extracting solvent and left the content in dark for 2 hours. Then after the absorbance was read at 505 nm, 511 nm, 523 nm and 535 nm for pelargonidine, delphinidine, peonidine and total anthocyanin contents. The values were expressed in mg/g fresh weight.

3.8.4.8 *Carotenoid content*:

The carotenoid content were estimated by using the method as described by Harborne(1976).

100mg petals were homogenized with 10ml (5 + 5ml) cold 80 per cent *ethanol in cold morter and pestle and centrifuged at 5000 rpm for* 10 minutes. Then, The absorbance of supernatant obtained was read directly at 447 nm, 453 nm and 460 nm for α- carotene, β- carotene and total carotenoid content, respectively against solvent as blank. The values were expressed in mg/g fresh weight.

3.8.4.9 *Ethylene production and respiration*:

Florets were excised from spikes at fully open, at the incipient wilting and at final wilting stage of 2nd floret and were placed with their bases in a 500ml glass jar containing distilled water. Florets were maintained at 25°C during the period ethylene was accumulating. After 4 hours, 1ml air samples were removed and ethylene concentration was determined by gas chromatography using a flame ionization detector. Ethylene measurements were expressed as nl/gfresh weight/h.

Changes in CO_2 evolution were determined by preparing a flower sampling in the same manner as for ethylene measurement. 1ml air samples were removed after 4 hours and CO_2 concentration was determined by gas chromatography with thermal conductivity detector and the CO_2 changes were expressed as µl/g fresh weight/h.

3.8.5 CORM PRODUCTION:

Various studies were made after lifting the plant with corms and cormels, when the shoot portion had dried down. Soil attached with the corms and cormels were carefully removed and kept in shade after having taken the fresh weight of corms and cormels.

3.8.5.1 *Number of corms per plant*:

From each treatment corm counts were made after having separation and collected from the plant.

3.8.5.2 *Diameter of corms(cm)*:

The measurement with regard to diameter of a corm was taken with the help of digital vernier callipers.

3.8.5.3 *Weight of corms (g)*:

Corms produced from each treatment were weighed for fresh weight after having been uprooted from the soil.

3.8.5.4 *Number of cormels per plant*:

From each treatment cormel counts were made after having separation and collected from the parent corm.

3.8.5.5 *Weight of cormels (g)*:

The cormels of each treatment were weighed for fresh weight and the data were recorded.

3.9 STATISTICAL ANALYSIS:

Statistical analysis was carried out for determining the significance of treatments, all the data recorded during course of experimentation were subjected to statistical analysis in Randomized Block Design and Completely Randomized Block Design (Panse and Sukhatme, 1995) by adopting analysis of variance method. The significance of different treatment effects was judged with the help of 'F' (variance ratio) test. The differences between the significant treatment means were tested against critical differences at 5 per cent level of significance where 'F' test was statistically significant.

3.9.1 Critical differences (C.D):

Critical differences were calculated by the following formula.

$$\text{C.D at 5\%} = \text{SEDM X 5\% Error d.f.}$$

$$\text{SEDM} = \sqrt{\frac{2 \text{ X Error MS}}{R}}$$

Where, 't' represents Fisher 't' tabulated value against error degree of freedom at 5% level of significance.

EXPERIMENTAL FINDINGS...

An endeavor has been made to elicit the influence of growth regulators either in combination with vesicular-arbuscular mycorrhiza (VAM) or alone in three ways viz. Presoaking, foliar and presoaking + foliar treatment on vegetative growth, flowering, physiological and bio-chemical changes during vase-life and corm production in *Gladiolus grandiflorus* L. cv. Jessica. The behaviour of cut spikes when kept under standard vase solution regarding quality characters has also been studied in this chapter are described as under.

The observation recorded at the successive stages of the crop growth were processed statistically are presented in tables and depicted in the form of graphs wherever its necessity at appropriate place. It is evident from the analysis of the data (table-1 to 46) that vegetative growth, flowering, physiological and bio-chemical responses during the vase-life and corm production in gladiolus and cut spikes were significantly influenced by the application of growth regulators either in combination with V.A.M or alone by all the three (presoaking, foliar and presoaking + foliar) adopted methods.

Experiments 1 : Studies on the effect of VAM and growth regulators on *Gladiolus grandiflorus* L. cv. Jessica.

4.1 VEGETATIVE GROWTH PARAMETERS:

4.1.1 *Number of days required for sprouting of corms*:

The data recorded on number of days required for sprouting of corms are presented in table-1. All the plant growth regulators in association with V.A.M treatment significantly reduced the number of days required for sprouting of corms over the control (19.30) except under T_8 and T_9. Minimum number of days taken for sprouting of corms (11.69) was observed under T_{12} followed by T_{13}, T_4, T_5, T_{10}, and T_{11} which did not differ significantly

Table-1 **Mean sprouting performance of different plant growth regulators in association with VAM treatment in *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	Days taken for sprouting of corms		Percent of sprouting of corms at 30 days after planting	
	1999-2000	2000-2001	1999-2000	2000-2001
T_1	19.30	17.59	58.33(49.83)	59.05(50.23)
T_2	13.83	13.98	81.25(64.15)	82.33(65.20)
T_3	14.77	14.08	77.08(61.42)	80.28(63.76)
T_4	12.48	12.80	83.33(65.95)	74.80(59.92)
T_5	12.99	12.89	81.25(64.38)	85.65(67.29)
T_6	16.82	16.19	81.25(64.36)	82.12(65.05)
T_7	17.18	16.67	79.16(62.95)	69.66(56.61)
T_8	18.46	17.01	77.08(61.48)	74.82(59.93)
T_9	18.83	17.13	68.75(56.02)	72.42(58.34)
T_{10}	13.15	13.24	81.25(64.36)	83.50(66.07)
T_{11}	13.43	13.25	83.33(65.95)	83.21(65.92)
T_{12}	11.69	11.54	83.33(65.95)	86.57(68.52)
T_{13}	12.34	12.72	77.08(61.46)	85.00(67.80)
CD (0.05)	1.19	1.59	3.61	3.82

among themselves. Kinetin in combination with V.A.M was found more effective than gibberellic acid in combination with V.A.M irrespective of the methods of application. Higher concentration of either of the two plant growth regulators in association with V.A.M did not vary significantly over the lower concentration. Similar results were observed during the second year of investigations.

4.1.2 *Percentage sprouting of corms at 30 days after planting*:

It is also clear from data in table-1 in comparison to the control (58.33), all plant growth regulators in association with V.A.M treatments significantly increased the percentage of sprouting of corms. However, maximum percentage sprouting of corm (83.33) was noticed under T_{12}, which was at par with T_2, T_4, T_5, T_6, T_{10}, and T_{11}. In general, higher concentration of plant growth regulators in association with V.A.M did not produce additional response over their lower doses except under T_3, T_7, T_8 and T_{13} during the year 1999-2000 and under T_6 and T_7 during the year 2000-2001 of the investigation.

4.1.3 *Plant height*:

The data recorded on plant height at 30 and 60 days after planting is presented in table-2. It is obvious from the data that plant height at 30 and 60 days after planting of corms were significantly increased by all the plant growth regulators in combination with V.A.M treatments as compared to the control (42.37cm and 60.72cm) except under T_6, T_7, T_8 and T_9, which shows that foliar application of plant growth regulators was not at all effective at 30 days after planting but was effective at 60 days after planting and differed significantly with control. However, T_{12} (54.32cm and 75.93cm) was the most effective in increasing plant height, which was at par with T_4 (51.30CM) but differed significantly with T_{10} (50.23cm and 70.68cm) and significantly higher than rest of the treatments. Application of kinetin in combination with V.A.M were more effective than the GA_3 in combination with V.A.M and the higher

Table-2 Mean growth performance of different plant growth regulators in association with VAM treatment in *Gladiolus grandiflorus* L. cv. Jessica.

Treatments	Plant height (cm)				Number of leaves				Leaf area (cm^2)	
	30 days		60 days		30 days		60 days			
	1999-2000	2000-2001	1999-2000	2000-2001	99-00	00-01	99-00	00-01	1999-2000	2000-2001
T_1	42.37	41.90	60.72	60.80	2.81	2.75	9.44	9.33	282.44	282.78
T_2	48.78	48.70	70.42	69.80	3.35	3.40	8.60	8.83	321.43	320.15
T_3	47.82	47.60	69.44	69.42	3.22	3.25	9.08	9.10	316.94	315.75
T_4	51.30	51.90	72.26	72.22	3.53	3.60	9.56	9.57	361.17	362.72
T_5	49.30	50.75	71.68	71.25	3.52	3.32	9.07	9.10	356.92	358.12
T_6	45.82	44.86	69.04	69.20	3.20	3.30	9.53	9.60	344.94	341.58
T_7	44.73	44.75	67.92	67.58	3.19	3.15	9.00	9.25	331.49	330.28
T_8	43.24	45.55	67.83	67.10	3.17	3.10	9.08	8.90	322.04	323.92
T_9	42.87	45.70	67.82	66.50	3.08	3.00	9.48	9.48	321.43	322.49
T_{10}	50.23	50.18	70.63	70.84	3.47	3.50	9.20	9.40	351.41	349.78
T_{11}	48.91	48.90	70.59	70.77	3.36	3.40	8.98	8.97	345.70	342.54
T_{12}	54.32	55.66	75.93	75.35	3.75	4.00	9.92	9.95	374.74	375.82
T_{13}	52.40	52.32	72.49	72.95	3.63	3.63	9.00	9.16	373.20	374.68
CD (0.05)	3.56	3.93	1.74	1.65	N.S	N.S	N.S	N.S	2.73	2.76

concentration of plant growth regulators in combination with V.A.M did not prove more effective at the lower concentration except T_{13}. Similar results were obtained during the second year of findings.

4.1.4 *Number of leaves:*

Number of leaves per plant when observed 30 and 60 days after planting did not vary significantly among the different treatment combination (table-2). However, maximum number of leaves at 30(3.75) and 60(9.92) days after planting under T_{12} were recorded whereas, it was minimum under T_1 (2.81) at 30days after planting and T_2 (8.60) at 60 days after planting.

4.1.5 *Leaf area*:

It is evident from data in table-2 that all the plant growth regulators in association with V.A.M treatment significantly increased the leaf area as compared to control (282.44cm^2). Combined application of Kinetin (presoak + foliar) in association with V.A.M i.e. T_{12} was most effective (374.74cm^2) in increasing the leaf area, which was at par with T_{13} (373.20cm^2). Kinetin in association with V.A.M was more effective in increasing the leaf area than GA_3 except T_9. Higher concentration of plant growth regulators in association with V.A.M did not prove more effective than the lower concentration but were also differed significantly with lower concentration except T_8 and T_9 in the second year of investigation.

4.2 FLOWERING PARAMETERS:

4.2.1 *Number of days required for spike initiation*:

The perusal of data regarding number of days required for spike initiation is given in the table-3. It is apparent from the data that number of days required for spike initiation were significantly reduced by all the

Table-3 **Mean performance of flowering characters of different plant growth regulators in association with VAM treatment in *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	Number of days required For Spike initiation 1999-2000	2000-2001	Total number of florets per spike 1999-2000	2000-2001
T_1	74.60	75.45	12.47	12.50
T_2	73.67	73.14	13.46	13.42
T_3	74.00	73.80	13.40	13.25
T_4	68.25	68.15	14.67	14.26
T_5	69.67	68.65	13.53	13.65
T_6	71.33	70.86	15.27	15.60
T_7	72.05	72.00	15.10	15.00
T_8	72.39	74.35	13.33	13.20
T_9	73.67	75.00	12.57	12.80
T_{10}	70.00	70.00	17.47	17.95
T_{11}	70.50	71.18	15.40	15.60
T_{12}	65.18	67.02	18.46	18.73
T_{13}	67.67	67.49	18.27	18.20
C.D (0.05)	**3.07**	**3.93**	**0.56**	**0.69**

treatments as compared to control (74.60) except T_2, T_3, T_7, T_8 and T_9. Minimum number of day's (65.18) for spike initiation was found in T_{12}, which was at par with T_{13} and differed significantly with T_{10}. Foliar application of kinetin in association with V.A.M at either of two concentration were not found effective, whereas, presoaking and presoaking + foliar proved better in enhancement of the spike initiation. However, GA_3 treatment in either of presoaking and foliar did not showed much response except T_{10} and T_{11}. Almost similar observation in the second year of the investigation was noticed.

4.2.2 *Total number of florets*:

It is inferred from the data in table-3 that number of florets significantly increased under all the treatments when compared with control which observed minimum number of florets (12.47) except under T_9 (12.57). Both the plant growth regulators and both the concentrations in association with V.A.M significantly increased the number of florets. However, lower concentration of both the plant growth regulators in association with V.A.M proved more effective than the higher concentration. Combined application of kinetin in combination with V.A.M i.e. T_{12} was the most effective (18.46) in increasing the number of florets, which was at par with T_{13}.

Almost similar observation in the second year of the investigation was noticed.

4.2.3 *Fresh weight of spike*:

It is clear from the data in table-4 that there was significant increase in the fresh weight of spike under all the treatments except T_9 (57.86g), which was at par with T_1 (56.20g). Maximum increase in the spike fresh weight was recorded under T_{12} (74.80g) followed by T_{13}, T_4, T_5, T_{10} and T_{11}. Kinetin in association with V.A.M was more effective in this regard than GA_3 in combination with V.A.M. There was no additional gain of the higher

Table-4 **Mean performance of spike fresh weight of different plant growth regulators in association with VAM treatment in *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	Fresh weight of cut spike		Daily fresh weight changes of cut spike		days to fresh weight changes	
	1999-2000	2000-2001	1999-2000	2000-2001	1999-2000	2000-2001
T_1	56.20	57.50	0.53	0.51	4.25	4.65
T_2	66.40	67.40	2.48	2.38	5.43	5.98
T_3	66.07	67.00	1.61	1.66	5.28	5.75
T_4	68.90	69.35	2.90	2.77	6.07	6.15
T_5	68.40	69.20	2.81	2.59	6.03	5.97
T_6	63.20	65.60	3.41	3.39	8.02	8.05
T_7	61.87	63.25	3.08	3.12	7.15	7.25
T_8	61.67	61.16	1.60	1.59	5.18	5.48
T_9	57.86	58.40	0.56	0.54	5.12	5.00
T_{10}	67.86	69.10	3.43	3.50	8.15	8.55
T_{11}	67.36	68.00	3.38	3.42	8.12	8.22
T_{12}	74.80	74.50	5.04	5.00	11.20	11.40
T_{13}	72.17	74.00	4.96	4.99	8.18	8.82
CD (0.05)	2.09	2.59	0.49	0.48	0.27	0.36

concentration of plant growth regulators than the lower ones. Similar results were observed during the second year of investigations.

Data regarding daily fresh weight changes in cut spike are given in table-4, revealed that in comparison to the control (0.53g), all the plant growth regulators in association with V.A.M treatments significantly increased the fresh weight except T_9 (0.56g). However, T_{12} was the most effective (5.04g) in doing so, which was at par with T_{13} and significantly higher than all other treatments. Lower concentration of plant growth regulators in association with V.A.M proved more effective than the higher rates.

It is also clear from the data in table-4 that number of days up to which fresh weight changes in spike continued was significantly enhanced by all the treatments in comparison to the control (4.25) but T_{12} (11.20) was the most effective. In general, higher rates of plant growth regulators did not prove more effective than the lower ones. Kinetin in combination with V.A.M proved considerably more effective than GA_3 with V.A.M.

Almost similar observation in the second year of the investigation was noticed.

4.3 CHANGES OCURRED IN PHYSIOLOGICAL PARAMETERS:

4.3.1 *Daily elongation of flower spike*:

Data recorded on elongation of flower spike is presented in table-5. It is apparent from data that the elongation of the cut flower spike and duration of elongation was significantly increased by all the treatments over the control (3.51mm and 5.11 days) except T_9 (3.78mm and 5.14 days). However, T12 was the most effective (14.85mm and 13.12 days) in this respect, immediately followed by T_{13}, T_{10}, T_{11}, T_6, T_7 and T_4. In general, kinetin in association with V.A.M proved more effective than GA_3 in combination with V.A.M, irrespective of the methods of application.

Almost similar observation in the second year of the investigation was noticed.

Table-5 **Mean performance of physiological characters of different plant growth regulators in association with VAM treatment in *Gladiolus grandiflorus* L. cv. Jessica.**

	Daily elongation of spike (mm)		days to elongation of spike	
Treatments	1999-2000	2000-2001	1999-2000	2000-2001
T_1	3.51	3.34	5.11	4.92
T_2	6.65	6.31	5.25	5.97
T_3	6.27	5.72	5.18	5.36
T_4	6.74	6.86	8.02	7.77
T_5	6.73	6.85	6.36	7.60
T_6	8.70	8.84	11.13	11.37
T_7	8.67	8.77	8.42	8.72
T_8	5.85	5.49	5.16	5.25
T_9	3.78	4.00	5.14	5.08
T_{10}	11.19	11.05	12.07	12.15
T_{11}	10.16	10.36	11.34	11.96
T_{12}	14.85	15.16	13.12	12.88
T_{13}	13.35	13.60	12.27	12.42
CD (0.05)	**1.10**	**1.55**	**0.36**	**0.38**

4.3.2 *Length of flower and Diameter of flower:*

It is evident from the data presented in table-6 shows that only presoaking + foliar application of the plant growth regulators in association with V.A.M were found effective in increasing the length of the floret over the control (9.02cm) and T_{12} (10.90cm) was most effective. In association with V.A.M presoaking or foliar application alone did not prove effective at all.

The data further exhibited that all the plant growth regulators in combination with V.A.M treatments except T_9 (7.95cm) significantly increased the flower diameter as compared to control (7.94cm) and T_{12} (10.62cm) was the most effective followed by T_{13} which was at par with rest of the treatments except T_1 and T_9. Higher concentration of plant growth regulators in association with V.A.M did not give response by either of the two plant growth regulators.

Almost similar observation in the second year of the investigation was noticed.

4.3.3 *Water uptake and water loss:*

It is apparent from data presented in table-7, there was a significant increase in water uptake as well as the water loss in comparison to the control (3.49ml and 3.00 ml) except T_9 (both water uptake and loss). T_{12} and T_{13} were at par with T_1. However, T_{12} was the most effective in increasing the water uptake (10.58ml) and reducing the water loss (3.05ml). Higher concentration did not prove much effect than the lower concentrations. While, in combination with V.A.M, kinetin application was more effective than GA_3 regardless of the method of application.

Almost similar observation in the second year of the investigation was noticed.

Table-6 **Mean performance of physiological characters of different plant growth regulators in association with VAM treatment in *Gladiolus grandiflorus* L. cv. Jessica.**

	Length of floret (cm)		Diameter of floret (cm)	
Treatments	**1999-2000**	**2000-2001**	**1999-2000**	**2000-2001**
T_1	9.02	8.92	7.94	8.02
T_2	9.33	9.59	9.06	9.17
T_3	9.20	9.40	9.00	9.02
T_4	9.40	9.65	9.38	9.26
T_5	9.37	9.60	9.34	9.24
T_6	9.43	9.75	9.52	9.42
T_7	9.40	9.73	9.39	9.34
T_8	9.10	9.12	8.85	8.94
T_9	9.05	9.00	7.95	8.06
T_{10}	9.48	9.80	9.77	9.88
T_{11}	9.47	9.78	9.57	9.39
T_{12}	10.90	11.47	10.62	10.68
T_{13}	9.75	9.95	9.89	9.92
CD (0.05)	**0.46**	**0.44**	**0.79**	**0.72**

Table-7 **Mean performance of water relation of spike of different plant growth regulators in association with VAM treatment in *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	Water Uptake (ml/spike/day) 1999-2000	2000-2001	Water Loss (ml/spike/day) 1999-2000	2000-2001
T_1	3.49	4.10	3.00	3.35
T_2	7.46	7.28	5.60	5.95
T_3	7.30	7.05	5.50	5.92
T_4	7.74	7.95	5.86	5.70
T_5	7.63	7.90	5.75	5.46
T_6	8.75	8.76	4.67	4.58
T_7	8.66	8.60	5.13	4.95
T_8	4.88	5.08	4.50	4.95
T_9	3.94	4.29	3.13	4.10
T_{10}	9.51	9.67	3.53	3.76
T_{11}	9.47	9.20	4.50	4.10
T_{12}	10.58	11.08	3.05	3.10
T_{13}	9.72	10.17	3.40	3.72
CD (0.05)	**0.48**	**0.83**	**0.45**	**0.51**

4.3.4 *Time required for opening of basal floret*:

The data presented in table-8 revealed that all the plant growth regulators in association with V.A.M treatments significantly increased the time required for opening of basal floret as compared to control (2.12). Although the differences between the lower and higher concentration of the plant growth regulators in association with V.A.M were not significant. Maximum days (5.46) for opening of basal floret were observed under T_{12} which was significantly higher than all other treatments except T_9. In association of V.A.M, effectiveness of combined application was followed by presoaking and foliar application.

Almost similar observation in the second year of the investigation was noticed.

4.3.5 *Days to senescence of basal floret*:

It is further clear from the data (table-8), days to senescence of basal floret increased significantly under all the treatments except T_9 (6.60) in comparison to control (6.26). However, T_{12} (10.25) was the most effective followed by T_{13} (9.32), which was at par with T_{10} (9.31) and T_{11} (9.23). Higher concentration of plant growth regulators did not effective over the lower concentration. Kinetin in association with V.A.M particularly under combined application and presoaking proved better than GA_3 in combination with V.A.M.

Almost similar observation in the second year of the investigation was noticed.

4.3.6 *Number of florets/spike open at the time of senescence of basal Floret*:

Number of opened florets/spike were significantly increased by various treatments except T_2 (4.15), T_3 (4.13), T_8 (4.13) and T_9 (4.11) were at par with T_1 (4.01) are presented in table-8. However, T_{12} (9.11) proved the

Table-8 *Mean performance of spike quality of different growth regulators in association with VAM treatment in Gladiolus grandiflorus* L. cv. Jessica.

Treatments	Time required for opening of basal floret(Days) 1999-2000	2000-2001	No. of days to senescence of basal floret 99-00	00-01	No. of florets/spike open at the time of senescence of basal floret 99-00	00-01	No. of floret/spike open before stem collapse 99-00	00-01	Longevity 1999-2000	2000-2001
T_1	2.12	1.92	6.26	6.25	4.01	3.92	4.12	4.20	7.17	7.20
T_2	3.36	3.96	7.16	6.91	4.15	4.50	6.12	6.25	9.34	9.66
T_3	3.30	3.57	7.10	6.62	4.13	4.41	5.18	5.35	9.17	9.62
T_4	4.37	4.40	7.17	7.72	5.17	5.07	7.29	7.36	13.23	12.10
T_5	4.15	4.20	7.13	6.70	4.68	4.65	7.18	6.52	11.07	11.24
T_6	4.53	4.75	8.68	8.50	6.20	6.52	8.06	7.99	14.28	14.20
T_7	4.47	4.62	8.15	8.29	5.19	5.37	7.58	7.88	14.12	13.95
T_8	3.25	3.15	7.13	6.60	4.13	4.16	4.20	4.30	9.12	9.04
T_9	2.20	2.45	6.60	6.50	4.11	4.05	4.15	4.25	7.70	7.85
T_{10}	4.74	4.95	9.31	9.45	6.46	6.74	8.15	8.10	15.12	15.35
T_{11}	4.54	4.80	9.23	9.36	6.25	6.38	8.08	8.06	14.92	14.40
T_{12}	5.46	5.66	10.25	10.48	9.11	9.40	9.12	9.45	17.05	17.85
T_{13}	4.84	5.06	9.32	9.49	7.76	7.95	8.18	8.30	16.95	17.10
CD (0.05)	0.33	0.46	0.50	0.77	0.21	0.25	0.24	0.44	0.29	0.42

best, which recorded significantly higher florets/spike than rest of the treatments. V.A.M in association with GA_3 as presoaking and foliar application of kinetin in association with V.A.M did not give any response.

Almost similar observation in the second year of the investigation was noticed.

4.3.7 *Number of florets/spike open before the stem collapse*:

Number of florets/spike open before the stem collapse is presented in table-8. All the treatments except T_8 (4.20) and T_9 (4.15) increased the number of opened florets per spike and this increase was the most pronounced under T_{12} followed by T_{13} (8.18), which was at par with T_{10} (8.15), T_{11} (8.08) and T_6 (8.06). V.A.M in combination with kinetin was more effective than V.A.M in association with GA_3, particularly under combined application and presoaking.

Almost similar observation in the second year of the investigation was noticed.

4.3.8 *Longevity*:

Longevity of the cut spike (table-8) was significantly increased by all the treatments, when compared with the control (7.17). However, T_{12} (17.05) was the most effective treatment which recorded an increased of more than 100 per cent in the longevity and was at par with T_{13}. Combined application of kinetin in association with V.A.M was followed by combined application of GA_3 in combination with V.A.M, foliar (GA_3 in combination with V.A.M), presoaking (kinetin in association with V.A.M) and presoaking (GA_3 in combination with V.A.M). Lower concentration of the plant growth regulators proved more effective.

Almost similar observation in the second year of the investigation was noticed.

4.4 BIO-CHEMICAL PARAMETERS:

4.4.1 *Enzymes*:

Activities of peroxidase and polyphenol oxidase under various treatments are given in the table-9. A critical evaluation of data showed that the activity of both the enzymes was significantly decreased over the control (88.00 and 143.04 units), although the magnitude of the activity was higher in case of polyphenol oxidase. Minimum enzyme activity 55.36 and 56.96 units was noticed under T_{12} which was significantly lower than all other treatments except T_4 (58.56 units) in case of peroxidase and T_{13} (58.56 units) in case of polyphenol oxidase. In general, higher concentration of plant growth regulators was directly related with the enzyme activity. Foliar application of kinetin in association with V.A.M did not affect the activity of either of the two enzymes when compared with the control.

4.4.2 *Protein*:

The perusal of data recorded on water-soluble protein, water insoluble protein and total protein are presented in table-10. It is apparent from data that water-soluble protein was significantly increased by all the plant growth regulators treatment in comparison to the control (9.18) that recorded the minimum water-soluble protein. However, T_{12} (41.35) was the most effective in enhancing the protein content that was significantly higher than all other treatments. In general, kinetin in association with V.A.M proved more effective than GA_3 in combination with V.A.M and higher concentration of plant growth regulators did not yield significant more response over the lower concentration. In contrast, both water insoluble and total protein significantly reduced under all the plant growth regulators in association with V.A.M treatment. The magnitude of reduction in water in-soluble protein 12.70 mg/g was observed under T_{12} and total protein (48.97mg/g) under T_7. Maximum water insoluble protein 50.16mg/g and total proteins were observed under T_1. Treatment combination viz. T_2, T_3, T_5, T_6, T_7, and T_{13} did not differed

Table-9 **Mean enzyme activity performance of different plant growth regulators in association with VAM treatment in fresh petal of *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	Peroxidase *1999-2000*	Polyphenol oxidase *1999-2000*
T_1	88.00	143.04
T_2	71.36	110.72
T_3	69.12	82.88
T_4	58.56	63.36
T_5	68.16	74.56
T_6	69.76	82.88
T_7	73.92	101.12
T_8	81.28	122.24
T_9	82.88	131.52
T_{10}	63.36	68.16
T_{11}	69.12	71.36
T_{12}	55.36	56.96
T_{13}	58.56	58.56
CD (0.05)	**6.63**	**15.96**

* Mean enzyme activity have been expressed as units**/gfw/h, except for peroxidase where it is units/gfw/min.

** I Unit = Change in 1.0 O.D for the enzyme peroxidase and Poly phenol oxidase at 430nm, respectively.

Table-10 Mean protein content of different plant growth regulators in association with VAM treatment in fresh petal of *Gladiolus grandiflorus* L. cv. Jessica.

Treatments	Water Soluble Protein (mg/g) 1999-2000	Water Insoluble Protein (mg/g) 1999-2000	Total Protein (mg/g) 1999-2000
T_1	9.18	50.16	59.34
T_2	17.65	34.24	51.89
T_3	17.11	35.70	52.81
T_4	21.15	30.08	51.23
T_5	20.32	33.23	53.55
T_6	26.03	26.32	52.35
T_7	24.94	27.50	52.44
T_8	13.27	35.70	48.97
T_9	13.27	42.21	55.48
T_{10}	32.67	16.51	49.18
T_{11}	30.77	26.27	57.04
T_{12}	41.35	12.70	54.05
T_{13}	35.58	15.94	51.52
CD (0.05)	**1.85**	**1.75**	**1.15**

significantly with each other. Increase in concentration of plant growth regulators over the lower concentration did not yield additional response.

4.4.3 *Nucleic acid*:

It is clear from the data in table-11 that there was a significant increase in RNA (98.23µg/g) and DNA (39.45µg/g) content except T_9 in comparison to the control (97.38µg/g and 38.79µg/g), respectively. Presoaking + foliar application of kinetin in combination with V.A.M treatment T_{12} proved the most effective (183.40µg/g RNA and 74.55µg/g DNA) which was significantly higher than all other treatments. In the order of effectiveness combined application of kinetin in association with V.A.M was followed by combined application of GA_3 in association with V.A.M, foliar kinetin in association with V.A.M and presoaking kinetin in association with V.A.M. Plant growth regulators at higher rates did not yield additional gain.

4.4.4 *Total carbohydrates and phenol*:

Majority of the plant growth regulators in association with V.A.M treatments was found effective in increasing the reducing sugar (table-12). T_{12} (21.34mg/g) was the most effective in this regard. Minimum reducing sugar (10.60mg/g) was observed under T_1 that was at par with T_3, T_8 and T_9. Kinetin in combination with V.A.M was found more effective than GA_3 in association with V.A.M except under foliar application of kinetin.

The data further revealed that there was significant decline in non-reducing sugar, when compared with control (6.18mg/g). Increase in concentration of plant growth regulators over their lower rate did not prove effective except under T_9 (5.89mg/g). However, T_{12} (1.91mg/g) recorded the minimum non-reducing sugar which was significantly lesser than all other treatments except T_{10} and T_{13}.

There was a considerable increase in the total sugar content under various treatments. T_6, T_{10}, T_{11}, T_{12}, and T_{13} significantly increased total sugar

Table-11 **Mean Nucleic acid content of different plant growth regulators in association with VAM treatment in fresh petal of *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	RNA (µg/g) 1999-2000	DNA (µg/g) 1999-2000
T_1	97.38	38.79
T_2	131.40	54.99
T_3	121.77	49.10
T_4	137.39	56.08
T_5	138.03	52.35
T_6	147.67	61.02
T_7	144.88	56.82
T_8	115.99	46.21
T_9	98.23	39.45
T_{10}	164.57	68.57
T_{11}	159.44	65.61
T_{12}	183.40	74.55
T_{13}	176.64	71.23
CD (0.05)	**1.04**	**1.75**

Table-12 **Mean carbohydrate, starch and phenol content of different plant growth regulators in association with VAM treatment in fresh petal of *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	Reducing Sugar (mg/g) 1999-2000	Non-Reducing Sugar (mg/g) 1999-2000	Total Carbohydrate (mg/g) 1999-2000	Starch (mg/g) 1999-2000	Phenol (mg/g) 1999-2000
T_1	10.60	6.18	17.10	1.09	1.95
T_2	13.01	3.36	16.55	2.31	1.49
T_3	11.51	3.69	15.39	2.14	1.55
T_4	14.56	3.35	18.09	2.51	1.41
T_5	14.08	3.69	17.96	2.51	1.43
T_6	16.81	3.29	20.27	2.88	1.33
T_7	14.72	3.14	18.02	2.79	1.37
T_8	10.60	3.78	14.58	2.14	1.60
T_9	10.60	5.89	16.80	1.67	1.69
T_{10}	19.06	2.27	21.45	3.38	1.28
T_{11}	17.19	2.83	20.17	3.05	1.32
T_{12}	21.34	1.91	23.35	3.54	1.14
T_{13}	19.59	2.15	21.85	3.44	1.21
CD (0.05)	**0.93**	**0.69**	**0.98**	**0.47**	**0.14**

content only in comparison to control (17.10mg/g). But T_{12} (23.35mg/g) was the most effective treatment followed by T_{13}, T_{10}, T_6 and T_{11}. Minimum (15.39mg/g) carbohydrates were observed under T_3.

The data (table-12) further exhibited that there was a considerable increase in starch and decrease in phenol content due to the various plant growth regulators in association with V.A.M treatments and this reduction was most pronounced under T_{12} (3.54mg/g starch and 1.14mg/g phenol) which was significant than all other treatments except T_{10} (1.28mg/g) T_{13} (1.21mg/g) in case of phenol content. Higher concentration of plant growth regulators could not give further response over their lower rate.

Maximum starch (1.09mg/g) and phenol (1.95mg/g) was however, recorded under T_1 (control).

4.4.5 *Anthocyanin content*:

Data regarding anthocyanin content are presented in table-13 signifies that all the treatments combination registered a significant increase in all the constituents (pelargonidine, delphinidine, peonidine) over the control (0.29mg/g pelargonidine, 0.34mg/g delphinidine and 0.42mg/g peonidine, respectively except under T_{11} in case of peonidine which was at par with control. However, Total anthocyanin increased significantly irrespective of the treatments. It was also observed that T_{12} showed most effective in enhancing all the constituents, which recorded 1.10mg/g pelargonidine, 0.80mg/g delphinidine, 0.92mg/g peonidine and 1.71mg/g total anthocyanin, respectively followed by T_{13} and T_{10}.

4.4.6 *Carotenoid*:

It is obvious from data in table-14 that all the plant growth regulators in association with V.A.M treatment significantly increased the α-carotene, β-carotene and total carotenoid in comparison to the control (0.79mg/g α-carotene, 0.67mg/g β-carotene and 0.81mg/g total carotenoid. Combined

Table-13 Mean anthocyanin content of different plant growth regulators in association with VAM treatment in fresh petal of *Gladiolus grandiflorus* L. cv. Jessica.

Treatments	Pelargonidine (mg/g) 1999-2000	Delphinidine (mg/g) 1999-2000	Peonidine (mg/g) 1999-2000	Total anthocyanin (mg/g) 1999-2000
T_1	0.29	0.34	0.42	0.62
T_2	0.40	0.46	0.52	0.82
T_3	0.39	0.45	0.52	0.77
T_4	0.43	0.49	0.56	0.85
T_5	0.42	0.47	0.54	0.85
T_6	0.55	0.55	0.63	0.97
T_7	0.50	0.55	0.62	0.91
T_8	0.34	0.43	0.50	0.77
T_9	0.33	0.39	0.45	0.63
T_{10}	1.00	0.63	0.72	1.05
T_{11}	0.67	0.59	0.66	1.00
T_{12}	1.10	0.80	0.92	1.71
T_{13}	1.02	0.70	0.77	1.21
CD (0.05)	**0.17**	**0.10**	**0.26**	**0.29**

Table-14 Mean carotenoid content of different plant growth regulators in association with VAM treatment in fresh petal of *Gladiolus grandiflorus* L. cv. Jessica.

Treatments	α-Carotene (mg/g) 1999-2000	β-Carotene (mg/g) 1999-2000	Total Carotenoid (mg/g) 1999-2000
T_1	0.79	0.67	0.81
T_2	0.98	0.94	1.37
T_3	0.82	0.75	1.34
T_4	1.33	1.27	1.42
T_5	1.28	1.26	1.39
T_6	1.39	1.33	1.42
T_7	1.34	1.30	1.42
T_8	0.87	0.90	1.21
T_9	0.81	0.82	1.19
T_{10}	1.84	1.74	2.53
T_{11}	1.71	1.65	1.75
T_{12}	2.74	2.02	3.54
T_{13}	2.68	1.81	2.69
CD (0.05)	0.11	0.06	0.08

application of plant growth regulators in combination with V.A.M was more effective than plant growth regulators in combination with V.A.M applied singly and T_{12} (2.74mg/g α-carotene, 2.02mg/g β-carotene, 3.54mg/g total carotenoid was the most effective in this respect which was at par with T_{13} (2.68mg/g α-carotene, 1.81mg/g β-carotene and 2.69mg/g total carotenoid). A striking feature of the findings was that presoaking with GA_3 at both the rates in association with V.A.M significantly reduced the α-carotene and β-carotene content as compared to the control.

4.5 pH:

Data recorded on pH changes over the initial standard value of 3:5 under various treatments are given in table-15. It can be inferred from the data that in comparison to the initial value, all the treatments including control registered a considerable increase. However, maximum pH 4.42 was noticed under T_1, which was significantly higher than all other treatments. Presoaking + foliar application in combination with V.A.M particularly under kinetin gave the most pronounced response with respect to reduction in pH in comparison to each other. Among the various treatment combinations, minimum pH was observed under T_{12} (3.47).

4.6 Ethylene evolution:

Periodic changes in ethylene concentration of the floret are given in table-17. It is clear from the data that there was a considerable increase in ethylene concentration on the phase 2 over the phase 1 of observation, with a subsequent decline on the phase 3, irrespective of the plant growth regulator treatments. All the treatments significantly reduced the ethylene evolution in comparison to the control, which recorded (1.96,3.95 and 2.98 nl/gfw/hr) of ethylene on phase1, phase2 and phase 3, respectively.

Table-15 **Mean pH of different plant growth regulators in association with VAM treatment during petal senescence in *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	Changes in pH 1999-2000	2000-2001
T_1	4.42	4.25
T_2	4.08	4.00
T_3	4.03	4.10
T_4	3.98	4.05
T_5	4.07	4.15
T_6	3.97	4.00
T_7	4.03	3.89
T_8	4.15	4.20
T_9	4.08	4.15
T_{10}	3.88	3.80
T_{11}	3.97	3.90
T_{12}	3.47	3.50
T_{13}	3.77	3.75
CD (0.05)	**0.19**	**0.16**

Table-17 Mean ethylene production (nl/gfw/hr) of different plant growth regulators in association with VAM treatment in fresh petal of *Gladiolus grandiflorus* L. cv. Jessica.

Treatments	Phase 1 1999-2000	Phase 2 1999-2000	Phase 3 1999-2000
T_1	1.96	3.95	2.98
T_2	1.46	2.49	2.04
T_3	1.50	2.70	2.15
T_4	1.42	2.30	1.72
T_5	1.45	2.45	1.90
T_6	1.30	2.12	1.60
T_7	1.35	2.17	1.69
T_8	1.60	3.03	2.17
T_9	1.74	3.08	2.48
T_{10}	1.20	1.91	1.38
T_{11}	1.26	2.09	1.46
T_{12}	0.90	1.37	1.07
T_{13}	1.16	1.80	1.30
C.D (0.05)	0.11	0.17	0.22

However, combined (presoak + foliar) application of kinetin i.e. T_{12} was the most effective in checking ethylene evolution, which recorded 0.90, 1.37 and 1.07nl/gfw/hr of ethylene on phase-1, phase-2, and phase-3, respectively. Higher concentration of either of the two plant growth regulators in combination with V.A.M did not show additional response over the lower rate in majority of the treatments except under T_9 and T_{13}.

4.7 Respiration:

Data presented in table 18 revealed that rate of respiration in comparison to the control (607.50, 885.32 and 793.35µl/gfw/hr on phase-1, phase-2 and phase-3, respectively), all thee plant growth regulators in association with V.A.M treatment significantly reduced the rate of respiration irrespective of the stage of observation. Magnitude of he reduction in rate of respiration was highest under T_{12} i.e. 276.00, 646.30 and 417.53 µl/gfw/hr on phase-1, phase-2 and phase-3, respectively which was significantly lower than all other treatments. Combined application of plant growth regulators in association with V.A.M, particularly kinetin was more effective followed by foliar application and presoaking. In general, higher concentration of either of two plant growth regulators in combination with V.A.M did not produce additional response irrespective of the stages of observation.

4.8 SENESCENCE PARAMETERS:

4.8.1 *Protein content*:

Data regarding water-soluble protein, water insoluble protein and total protein content during petal senescence is presented in table-19. It can be inferred from the data that all the treatment combinations as compared to the control i.e. 29.48mg/g and 38.48mg/g, whereas the water insoluble protein

Table-18 **Mean respiration production (µl/gfw/hr) of different plant growth regulators in association with VAM treatment in fresh petal of *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	Phase 1 1999-2000	Phase 2 1999-2000	Phase 3 1999-2000
T_1	607.50	885.32	793.35
T_2	486.66	859.21	668.45
T_3	515.60	874.30	720.85
T_4	550.65	873.60	725.90
T_5	582.30	884.67	750.67
T_6	385.67	748.08	600.97
T_7	405.00	760.30	618.25
T_8	437.00	820.45	628.50
T_9	468.00	840.86	634.75
T_{10}	321.00	708.43	520.60
T_{11}	332.33	710.90	597.74
T_{12}	276.00	646.30	417.53
T_{13}	299.00	667.35	435.14
CD (0.05)	**3.80**	**8.93**	**9.61**

Table-19 Mean protein content of different plant growth regulators in association with VAM treatment during petal senescence in *Gladiolus grandiflorus* L. cv. Jessica.

Treatments	Water Soluble Protein (mg/g) 1999-2000	Water Insoluble Protein (mg/g) 1999-2000	Total Protein (mg/g) 1999-2000
T_1	29.48	9.00	38.48
T_2	42.50	4.25	46.75
T_3	41.30	4.44	45.74
T_4	42.50	2.99	45.49
T_5	42.50	3.27	45.77
T_6	43.69	2.83	46.52
T_7	43.69	2.93	46.62
T_8	39.35	6.95	46.30
T_9	38.91	7.62	46.53
T_{10}	45.49	0.88	46.37
T_{11}	45.49	1.10	46.59
T_{12}	50.43	0.12	50.55
T_{13}	49.08	0.37	49.45
CD (0.05)	**0.73**	**0.43**	**0.56**

showed a significant reduction under all the treatments in comparison to the control (9.00mg/g). However, maximum water-soluble protein (50.43mg/g) and total protein (50.55mg/g) were observed under T_{12}. In general, presoaking + foliar application in combination with V.A.M were more effective than foliar or presoaking alone. Water insoluble protein were noticed to be minimum under T_{12} (0.12mg/g) which was significantly lesser than all other treatments except T_{13} (0.37), where it was at par.

4.8.2 *Nucleic acid content*:

A critical evaluation of data presented in table-20 revealed that there was significant increase in RNA and DNA content due to all the V.A.M with plant growth regulator treatments as compared to the control (93.73μg/g RNA and 37.64μg/g DNA). Lower concentration of plant growth regulator in association with V.A.M proved more effective than the higher doses. Maximum increase in RNA (265.47μg/g) and DNA (109.25μg/g) was observed under T_{12}, which was significantly higher than all other treatment combinations. Combined application of plant growth regulators in association with V.A.M were the most effective followed by foliar application and presoaking except in case of T_8 (155.69μg/g) and T_9 (128.40μg/g).

4.8.3 *Total carbohydrate, starch and Phenol content*:

It is apparent from the data presented in table-21 showed that in comparison to the control (6.91mg/g) there was a significant increase in reducing sugar under all the treatment combination. However, T_{12} (15.02mg/g) was the most effective in increasing the reducing sugar, which was significantly higher than all other treatments except T_{13} (14.54mg/g).

The data further exhibited that non-reducing sugar and total carbohydrate decreased significantly under all the treatments. Maximum non-reducing sugar (26.07mg/g) and total sugar (34.35mg/g) were observed

Table-20 **Mean Nucleic acid content of different plant growth regulators in association with VAM treatment during petal senescence in *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	RNA (µg/g) 1999-2000	DNA (µg/g) 1999-2000
T_1	93.73	37.64
T_2	170.78	73.61
T_3	163.39	66.96
T_4	177.20	74.77
T_5	170.78	68.03
T_6	188.75	78.65
T_7	187.79	74.82
T_8	155.69	64.33
T_9	128.40	52.20
T_{10}	205.77	89.46
T_{11}	199.03	84.69
T_{12}	265.47	109.25
T_{13}	231.12	93.95
CD (0.05)	**2.18**	**2.12**

Table-21 **Mean carbohydrate, starch and phenol content of different plant growth regulators in association with VAM treatment during petal senescence in *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	Reducing sugar (mg/g) 1999-2000	Non-Reducing sugar (mg/g) 1999-2000	Total Carbohydrate (mg/g) 1999-2000	Starch (mg/g) 1999-2000	Phenol (mg/g) 1999-2000
T_1	6.91	26.07	34.35	0.46	2.32
T_2	10.04	11.22	21.85	0.88	1.92
T_3	8.19	14.22	23.16	0.87	1.92
T_4	11.48	7.71	19.60	0.95	1.76
T_5	11.24	10.19	21.97	0.92	1.76
T_6	12.45	6.34	19.12	1.08	1.67
T_7	12.45	7.65	20.50	1.00	1.67
T_8	7.79	14.67	23.23	0.65	2.14
T_9	7.38	20.32	28.77	0.60	2.20
T_{10}	13.97	5.63	19.90	1.13	1.41
T_{11}	12.93	6.34	18.62	1.11	1.58
T_{12}	15.02	3.48	18.60	1.34	1.17
T_{13}	14.54	4.11	18.14	1.19	1.39
CD (0.05)	**0.56**	**0.42**	**0.68**	**0.31**	**0.28**

under T_1, whereas these were lowest under T_{12} (3.48mg/g and 18.60mg/g). There was no additional response of higher concentration of either of the two-plant growth regulator over their lower concentration any of the three parameters.

It is further clear from the data in table-21 that both starch and phenol content showed significant increase under all the treatment combinations except T_8 and T_9 in case of starch content only. Minimum starch (0.46mg/g) and maximum phenol content (2.32mg/g) was observed under control, which was significantly higher than all other treatments except T_8 (0.65mg/g and 2.14mg/g) and T_9 (0.60mg/g and 2.20mg/g) where it was at par. Presoaking + foliar application in association with V.A.M particularly under kinetin resulted maximum starch and minimum phenol content. Maximum starch (1.34mg/g) and phenol (1.17mg/g) was noticed under T_{12}.

4.8.4 *Anthocyanin content*:

It is evident from the data in table-22 that there was a considerable increase in pelargondine, delphinidine, peonidine and total anthocyanin content but in comparison to the control except T_9 in case of peonidine, the differences were not significant under T_9 for pelargonidine, under T_2 and T_9 for delphinidine, under T_9 for peonidine. It is also clear that the foliar application of kinetin at lower rates could prove effective. Maximum pelargonidine (0.82mg/g), delphinidine (0.70mg/g), peonidine (0.88) and total anthocyanin (1.12mg/g) observed under T_{12}, whereas, these were lowest under control.

4.8.5 *Carotenoid content*:

The data regarding carotenoid content is given in table-23. It is quit obvious that α and β-carotene and total carotenoid content was significantly increased under T_4, T_5, T_6, T_7, T_{10}, and T_{12}. T_4, T_6, T_7, T_{10}, and T_{12} only for α-

Table-22 Mean anthocyanin content of different plant growth regulators in association with VAM treatment during petal senescence in *Gladiolus grandiflorus* L. cv. Jessica.

Treatments	Pelargonidine (mg/g) 1999-2000	Delphinidine (mg/g) 1999-2000	Peonidine (mg/g) 1999-2000	Total anthocyanin (mg/g) 1999-2000
T_1	0.08	0.12	0.10	0.32
T_2	0.28	0.16	0.21	0.52
T_3	0.20	0.18	0.18	0.47
T_4	0.29	0.24	0.29	0.74
T_5	0.25	0.25	0.26	0.50
T_6	0.35	0.28	0.34	0.91
T_7	0.34	0.26	0.29	0.90
T_8	0.13	0.17	0.14	0.40
T_9	0.12	0.12	0.10	0.37
T_{10}	0.69	0.37	0.45	1.02
T_{11}	0.36	0.34	0.39	0.99
T_{12}	0.82	0.70	0.88	1.12
T_{13}	0.75	0.39	0.45	1.03
CD (0.05)	**0.04**	**0.04**	**0.03**	**0.07**

Table-23 Mean carotenoid content of different plant growth regulators in association with VAM treatment during petal senescence in *Gladiolus grandiflorus* L. cv. Jessica.

Treatments	α-Carotene (mg/g) 1999-2000	β-Carotene (mg/g) 1999-2000	Total Carotenoid (mg/g) 1999-2000
T_1	0.65	0.19	0.75
T_2	0.30	0.56	1.10
T_3	0.39	0.22	0.85
T_4	0.98	0.98	1.00
T_5	0.91	0.75	0.99
T_6	1.10	0.98	1.12
T_7	0.92	0.94	0.93
T_8	0.56	0.23	0.92
T_9	0.53	0.21	0.82
T_{10}	1.19	0.98	1.22
T_{11}	0.87	0.86	0.90
T_{12}	1.20	1.14	1.27
T_{13}	0.83	0.67	0.89
C.D (0.05)	**0.26**	**0.21**	**0.18**

carotene, under T_2, T_4, T_5, T_6, T_7, T_{10}, T_{11}, T_{12} and T_{13} for β-carotene and under T_2, T_4, T_5, T_6, T_{10}, and T_{12} for total carotenoids were significant and rest of the treatment combination were at par with the control, which recorded 0.65mg/g α-carotene, 0.19mg/g β-carotene and 0.75mg/g total carotenoids, respectively. On the other hand, maximum α-carotene (1.20mg/g), β-carotene (1.14mg/g) and total carotenoids (1.27mg/g) were observed under T_{12}.

4.9 Corm Production:

The data presented in table-16 indicates that number of corms per plant was significantly increased only under T_4, T_5, T_6, T_7, T_{10}, T_{11}, T_{12}, and T_{13} which did not differed significantly among themselves.

Number of corms per plant were also increased significantly over the control (6.85) under T_4, T_5, T_6, T_8, T_{10}, T_{11}, T_{12} and T_{13}, all of which were at par with each other. There was no significant difference between the two concentrations of either of the two plant growth regulators.

There was a significant increase in the mean weight of corm and cormels and diameter of the corm under all the treatment combinations. However, maximum weight of corm (26.77g), weight of cormels (19.25g) and diameter of corm (5.25cm) was observed under T_{10} followed by T_{11}, T_4, T_{12}, T_6, and T_{13} for weight of corms, T_4, T_5, T_{11}, T12 for weight of cormels and T_4, T_5, T_6, T_8, T_{11}, T_{12}, and T_{13} for diameter of corm. Minimum number of corms, cormels weight of corms, weight of cormels and corm diameter were noticed under T_1 (control). In general, presoaking with GA_3 in combination with V.A.M at both the rates did not prove effective except for the mean weight of the corms. Most of the treatments involving presoaking + foliar application of plant growth regulators did not produce variable response in comparison to each other.

Table-16 **Mean performance of corms of different plant growth regulators in association with VAM treatment in fresh petal of *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	Number of Corms		Number of Cormels		Mean weight of corms(g)		Mean weight of cormels(g)		Diameter of corms(cm)	
	1999-2000	2000-2001	99-00	00-01	99-00	00-01	99-00	00-01	1999-2000	2000-2001
T_1	4.50	4.42	6.85	6.75	15.96	16.42	14.69	14.68	4.15	4.30
T_2	4.62	4.65	6.95	7.00	21.89	22.54	15.81	15.90	4.93	4.65
T_3	4.60	4.50	6.94	6.94	21.30	20.82	15.37	15.34	4.82	4.72
T_4	5.27	5.52	7.56	7.92	26.09	26.75	18.92	19.12	5.14	5.20
T_5	5.23	5.25	7.44	7.69	25.13	25.72	18.89	18.89	5.10	5.16
T_6	5.14	5.09	7.31	7.38	23.85	24.00	18.21	17.56	5.06	5.00
T_7	5.08	5.02	7.15	7.25	23.79	23.25	16.54	16.95	4.95	4.98
T_8	4.79	4.75	7.31	7.15	23.25	23.20	16.34	16.56	5.02	4.80
T_9	4.64	4.60	7.21	7.06	22.19	22.95	15.83	15.92	4.94	4.77
T_{10}	5.31	5.56	7.75	7.95	26.77	26.85	19.25	19.26	5.23	5.27
T_{11}	5.23	5.50	7.66	7.80	26.46	26.80	18.92	19.18	5.15	5.25
T_{12}	5.19	5.25	7.35	7.58	25.66	24.90	18.78	18.51	5.14	5.18
T_{13}	5.15	5.20	7.32	7.40	24.07	24.48	18.30	18.20	5.07	5.07
CD (0.05)	0.36	0.28	0.42	0.34	1.07	1.46	0.65	0.30	0.23	0.33

Experiment 2 : Studies on the effect of pre-soaking and foliar spray of growth regulators on *Gladiolus grandiflorus* L. cv. Jessica.

4.10 VEGETATIVE GROWTH PARAMETERS:.

4.10.1 *Number of days required for sprouting of corms*:

It is evident from data presented in table-24 that the number of days required for sprouting were significantly reduced by both presoaking as well as presoaking + foliar application of both the plant growth regulators in comparison to the control (19.76). However, minimum number of days for sprouting (11.44) was recorded under T_{11}, which was at par with T_2, T_3, T_4 and T_{10}. Higher concentration either of the two plant growth regulator either alone or in combination did not produce the additional response over the lower concentration. In general there was no effect of foliar application of either of the two plant growth regulators on the days required for sprouting. The data followed a similar trend during the second year of investigation.

4.10.2 *Percentage of sprouting at 30 days after planting*:

The data presented in table-24 revealed that per cent sprouting of the corms at 30 days after planting was significantly enhanced by both GA_3 and kinetin either alone (pre-soaking) or in combination (pre-soaking + foliar). On the other hand there was no effect of foliar application of either of the two plant growth regulator. Maximum percentage of sprouting was observed under T_{11} (89.58) that was at par with T_3 (87.50). Higher concentration of either of the plant growth regulators could not produce additional gain over their lower concentration, except under T_{11}. However, minimum sprouting was observed under T_9 (76.08), which was at par with T_1, T_6, T_7, and T_8. Almost similar results were obtained during second year of investigation.

Table-24 Mean sprouting performance of different treatments of plant growth regulators in *Gladiolus grandiflorus* L. cv. Jessica.

	Number of days required for sprouting		Percentage of sprouting at 30 days after planting.	
Treatments	**1999-2000**	**2000-2001**	**1999-2000**	**2000-2001**
T_1	19.76	19.53	79.17(62.95)	77.01(61.69)
T_2	12.57	13.67	85.42(67.68)	84.96(67.97)
T_3	11.48	10.65	87.50(69.36)	87.88(69.87)
T_4	12.36	12.40	83.33(66.00)	83.22(65.89)
T_5	13.04	13.35	85.42(67.66)	87.47(69.49)
T_6	18.63	18.74	77.92(61.59)	78.42(62.35)
T_7	19.03	18.65	76.83(61.33)	79.86(63.71)
T_8	19.95	19.92	78.33(62.95)	76.05(60.98)
T_9	19.19	19.88	76.08(60.70)	75.62(60.53)
T_{10}	11.93	11.30	83.33(66.09)	85.40(67.69)
T_{11}	11.44	11.50	89.58(71.23)	91.27(72.94)
T_{12}	12.69	12.89	84.16(66.58)	82.82(65.56)
T_{13}	13.00	13.50	85.50(67.62)	84.20(66.62)
C.D (0.05)	**1.20**	**1.41**	**3.90**	**4.17**

4.10.3 *Plant height*:

The data recorded on plant height under various treatments at 30 and 60 days after planting are presented in table-25. A critical evaluation of the data revealed that the plant height was significantly increased by application of GA_3 either alone or in combination as compared to the control (51.38cm and 71.67cm). In contrast, kinetin either alone or in combination reduced plant height in comparison to the control both at 30 and 60 days after planting. The effect of kinetin was much pronounced when applied in combination of pre-soaking + foliar. However, maximum plant height (59.61cm and 83.92cm at 30 and 60 days after planting, respectively was observed under T_{10}, whereas it was minimum (42.43cm and 67.41cm at 30 and 60 days after planting, respectively) under T_{12}. The data followed a similar trend during the subsequent year.

4.10.4 *Number of leaves /plant at 30 and 60 days after planting*:

The data presented in table-25 also exhibited that none of the treatments affected the number of leaves per plant when observed at 30 and 60 days after planting. However, the number of leaves was found maximum (3.80 and 9.20 at 30 and 60 days after planting, respectively under T_4, whereas it was minimum 3.00 and 8.33 under control (T_1).

4.10.5 *Leaf area*:

It is quite obvious from the data (table-25) that the leaf area was significantly increased by GA_3 application as either pre-soaking or foliar application or in combination in comparison to the control (401.38cm^2). Maximum leaf area (517.16cm^2) was observed under T_{11}, which was at par with T_{10}. Higher rates of GA_3 did not give additional response. Application of kinetin as pre-soaking, foliar and pre-soaking + foliar significantly reduced the leaf area over the control. Minimum leaf area (320.48cm^2) was recorded in case of T_{13} which was at par with T_{12} and significantly lesser than all the

Table-25 **Mean growth performance of different treatments of plant growth regulators in *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	Plant Height (cm)				Number of Leaves				Leaf Area (cm^2)	
	30 days		60 days		30 days		60 days			
	1999-2000	2000-2001	99-00	00-01	99-00	00-01	99-00	00-01	1999-2000	2000-2001
T_1	51.38	52.02	71.67	70.75	3.65	3.75	9.58	9.50	401.38	377.08
T_2	56.99	56.36	78.71	79.88	3.61	3.67	8.78	8.75	496.68	487.28
T_3	58.03	58.64	77.97	78.25	3.53	3.50	9.53	9.75	498.32	491.32
T_4	48.53	47.35	68.31	67.82	3.50	3.80	9.20	9.27	343.81	344.61
T_5	48.42	48.90	67.68	67.68	2.97	3.00	8.33	8.15	338.54	337.04
T_6	50.84	51.72	72.78	73.64	3.07	3.20	9.21	9.10	504.82	502.62
T_7	49.72	50.67	73.69	74.20	3.42	3.35	9.51	9.67	508.90	509.71
T_8	50.78	49.23	68.17	69.82	3.58	3.50	9.66	9.32	351.46	351.86
T_9	49.77	50.80	66.88	68.05	3.25	3.15	9.23	9.20	342.47	344.87
T_{10}	59.61	59.22	83.92	82.93	3.45	3.60	9.24	9.36	513.55	518.45
T_{11}	57.40	58.31	82.70	84.46	3.14	3.22	9.43	9.18	517.16	515.94
T_{12}	42.43	41.08	67.41	66.85	3.30	3.32	9.33	9.30	321.47	322.07
T_{13}	43.63	43.65	67.89	68.37	3.17	3.10	8.75	8.80	320.48	322.84
C.D (0.05)	**2.22**	**2.62**	**2.76**	**2.90**	**N.S**	**N.S**	**N.S**	**N.S**	**6.23**	**6.42**

treatments. In general, higher concentration of kinetin singly or in combination could not prove more effective.

Almost similar observation in the second year of the investigation was noticed.

4.11 FLOWERING PARAMETERS:

4.11.1 *Number of days required for spike initiation*:

The data recorded on number of days required for spike initiation is presented in table-26. It is apparent from data that application of GA_3 as pre-soaking, foliar and pre-soaking + foliar significantly advanced spike initiation as compared to the control (71.60). Higher concentration of GA_3 when applied any of the three methods of application further reduced the days required for spike initiation over their lower concentration, though the differences were non-significant. However, minimum number of day's (63.80) was observed under T_{11}, which was at par with T_3 and T_{10}.

It is further clear from data that kinetin applied as pre-soaking did not affect the number of days required for spike initiation at either of the two doses, as compared to the control. But application of kinetin either as foliar or pre-soaking + foliar considerably reduced the days required for spike initiation. Maximum number of day's (72.57) was recorded under T_4, which was at par with T_1 and T_5. Similar results were observed in second year of investigation.

4.11.2 *Number of florets/spike*:

A critical examination of the data (table-26) showed that number of florets/spike were significantly increased by the application of GA_3 at either of three application in comparison to the control (15.27). In contrast, application of kinetin considerably reduced the number of florets per spike in comparison to the control, although the differences were significant only

Table-26 **Mean performance of flowering characters of different plant growth regulators treatment in *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	Number of days required For Spike initiation 1999-2000	2000-2001	Total number of florets per spike 1999-2000	2000-2001
T_1	71.60	70.40	15.27	14.65
T_2	66.10	64.00	18.73	19.20
T_3	64.97	65.25	18.38	18.75
T_4	72.57	72.30	14.86	14.78
T_5	70.67	70.98	14.27	14.80
T_6	68.20	67.50	18.27	19.45
T_7	67.80	68.16	17.53	17.80
T_8	70.43	68.50	14.40	13.69
T_9	69.05	70.32	14.26	14.60
T_{10}	64.63	62.40	19.47	19.22
T_{11}	63.80	63.20	18.13	18.98
T_{12}	68.74	68.02	14.94	14.36
T_{13}	67.20	67.55	13.31	12.67
C.D (0.05)	**2.05**	**2.66**	**1.66**	**1.32**

under T_{13}, which recorded the minimum number of florets per spike (13.31). However, maximum number of florets per spike was obtained in case of T_{10} (pre-soaking + foliar GA_3 100ppm).

Almost similar observation in the second year of the investigation was noticed.

4.11.3 *Fresh weight of spike at the time of harvesting*:

It is apparent from the data (table-27) that fresh weight of spike at the time of cutting was significantly increased by GA_3 either alone or in combination in comparison to the control (68.90). But higher rates of GA_3 did not prove more effective as compared to the lower concentration when applied either as pre-soaking or foliar. However, combination of pre-soaking + foliar application particularly at the higher rates proved the most effective in enhancing the spike weight. Maximum spike weight 79.40g was noticed under T_{11}, whereas, it was minimum (60.45g) under T_{13}.

There was a significant decrease in fresh weight of the spike with the application of kinetin at any of the three methods particularly at higher rates. Similar trend was observed during subsequent year of investigation.

4.11.4 *Daily fresh weight changes of cut spike*:

The data given in table-27 further revealed that GA_3 application significantly increased fresh weight of spike when applied at any of the three methods in comparison to the control (4.43g). However, pre-soaking + foliar application at higher rates proved the most effective although the differences between the higher and lower rates were did not differed significantly. Maximum increase in daily fresh weight of the spike (6.30g) was obtained under T_{11}, which was at par with T_{10}. At a particular stage of application none of the treatments exhibited significant differences. Application of kinetin was found to decrease the daily fresh weight of the spike when applied at any of the three methods. But pre-soaking of the corms with kinetin affected the spike weight most adversely. Minimum daily spike weight changes were

Table-27 Mean performance of spike fresh weight of different plant growth regulators treatment in *Gladiolus grandiflorus* L. cv. Jessica.

Treatments	Fresh weight of spike at the time of cutting (g) 1999-2000	2000-2001	Daily fresh weight changes of cut spike (g) 1999-2000	2000-2001	days to fresh weight changes 1999-2000	2000-2001
T_1	68.90	68.00	4.43	4.23	8.10	8.15
T_2	73.00	72.08	5.10	5.19	10.40	10.35
T_3	74.70	74.95	4.99	5.04	9.11	8.95
T_4	67.40	66.80	1.15	1.05	5.48	5.12
T_5	65.20	64.53	1.10	1.15	5.87	6.02
T_6	69.00	69.85	5.51	5.18	10.55	10.48
T_7	68.50	69.00	5.38	5.58	10.63	10.61
T_8	66.50	64.20	2.52	2.72	5.95	6.93
T_{10}	77.50	76.56	6.18	6.31	6.06	7.42
T_{11}	79.40	78.48	6.30	6.28	11.95	12.10
T_{12}	62.30	61.10	3.10	3.15	6.75	5.81
T_{13}	60.45	58.95	3.42	3.62	7.25	6.00
C.D (0.05)	1.64	1.45	0.31	0.37	0.26	0.30

observed under T_5 (1.10g). The data followed similar result during second year of the investigation.

4.11.5 *Days to fresh weight changes of cut spike:*

Duration of the fresh weight changes (table-27) was significantly increased by GA_3 application at all the methods, as compared to the control (8.10 days). However, the maximum duration (11.95 days) upto that the fresh weight changes occurred was obtained under T_{11}, which was at par with T_{10}. Combination of pre-soaking and foliar application, at rate of GA_3 proved the most effective in enhancing the duration of fresh weight changes. After this the fresh weight of the spike continued to decrease till the termination of the vase-life. Application of kinetin at all the three methods significantly reduced the duration of fresh weight changes of the spike as compared to the control as well as GA_3. However, application of kinetin as pre-soaking was the most harmful. The minimum duration of fresh weight changes in the spike was recorded under T_4 (5.48 days).

Almost similar observation in the second year of the investigation was noticed.

4.12 PHYSIOLOGICAL PARAMETERS:

4.12.1 *Elongation of flower spike:*

The data regarding daily elongation of spike is presented in table-28. It is apparent from the data that spike elongation was significantly enhanced by GA_3 application irrespective of the method of treatments. Combined application of GA_3 (pre-soaking + foliar) produced the most beneficial effect. In general, higher rates of GA_3 were not much effective over the lower doses. However, maximum daily spike elongation was obtained under T_{10} that was at par with T_7, T_{11}.

Table-28 **Mean performance of physiological characters of different plant growth regulators treatment in *Gladiolus grandiflorus* L. cv. Jessica.**

	Daily elongation of spike (mm)		days to elongation of spike	
Treatments	**1999-2000**	**2000-2001**	**1999-2000**	**2000-2001**
T_1	5.71	5.51	6.06	6.10
T_2	6.83	6.70	11.70	11.47
T_3	6.95	6.93	11.35	11.17
T_4	3.80	3.68	9.75	9.68
T_5	3.38	3.63	7.23	8.36
T_6	6.00	5.95	13.90	13.93
T_7	6.77	6.87	13.28	13.36
T_8	4.20	4.38	10.22	10.32
T_9	4.86	4.94	10.05	10.10
T_{10}	7.54	7.69	14.50	14.50
T_{11}	7.29	7.26	14.10	14.20
T_{12}	4.30	4.37	13.25	13.08
T_{13}	4.28	4.26	13.12	13.05
CD (0.05)	**0.50**	**0.59**	**0.28**	**0.31**

Initial Spike Length: 53.50cm.

It is also evident from the data that application of kinetin significantly reduced the daily spike elongation and the higher doses of kinetin proved inhibitory to this. Minimum elongation of the spike (3.38mm) was recorded under T_5 against 5.71mm in control. Similar results were observed during the subsequent year of the investigation.

4.12.2 *Days to elongation of cut spike*:

The critical observation of the data (table-28) also revealed that the application of GA_3 at all three methods significantly increased the number of day's upto which elongation of the cut spike continued over the control (7.23days). After this elongation of the cut spike ceased. Maximum duration (14.50 days) of elongation of the spike was obtained under T_{10}, which was at par with T_{11} and immediately followed T_6, T_7, T_{12} and T_{13}. However, minimum duration of spike elongation (6.06 days) was recorded under T_1. Higher concentration of either of the two plant growth regulator did not play any role in increasing the duration of the elongation of the cut spike. In contrast, pre-soaking with higher rate of kinetin significantly decreased the duration of elongation of the cut spike in comparison to all other treatments except control.

Similar results were observed during second year of the investigation.

4.12.3 *Length of flower*:

A perusal of data (table-29) revealed that in general, application of either GA_3 or kinetin as pre-soaking or foliar application was not effective in improving the length of flower in comparison to control (9.64cm) except T_5 and T_8 (10.10cm), that produced significantly higher length. However, combination of pre-soaking and foliar application significantly increased flower length irrespective of the growth regulator. Maximum length of flower (10.80cm) was observed under T_{13}, which was the most effective of all the treatments. Higher rate of either GA_3 or kinetin did not give additional response over the lower ones, Minimum flower length (9.38cm) was noticed

Table-29 **Mean performance of physiological characters of different plant growth regulators treatment in *Gladiolus grandiflorus* L. cv. Jessica.**

	Length of floret (cm)		Diameter of floret (cm)	
Treatments	**1999-2000**	**2000-2001**	**1999-2000**	**2000-2001**
T_1	9.64	9.42	8.87	8.85
T_2	9.95	9.96	10.04	10.06
T_3	9.87	9.81	9.89	9.90
T_4	9.82	9.85	10.03	10.05
T_5	10.10	10.19	9.82	9.90
T_6	9.80	9.68	10.10	10.10
T_7	9.73	9.87	9.70	9.80
T_8	10.10	10.90	10.11	10.02
T_9	9.38	9.23	10.05	10.07
T_{10}	10.07	10.05	10.43	10.42
T_{11}	10.32	10.41	10.52	10.54
T_{12}	10.65	10.74	10.22	10.21
T_{13}	10.80	10.95	10.44	10.42
CD (0.05)	**0.37**	**0.65**	**0.08**	**0.08**

in case of T_9. The data followed almost similar trend during the second year of the study.

4.12.4 *Diameter of flower*:

All the treatments significantly increased the flower diameter (table-29) when compared with the control (8.87cm) which produced minimum diameter. However, maximum flower diameter (10.52cm) was recorded under T_{11}, which was significantly higher than all other treatments except T_{10} (10.43cm) and T_{13} (10.44cm), where it was at par. Higher concentration of both the plant growth regulator when applied either as pre-soaking or foliar spray resulted in a reduction in flower diameter. But the combined application (pre-soaking + foliar) at higher rate was more effective in enhancing flower diameter as compared to the lower concentration. Similar results were obtained during subsequent year of investigation.

4.12.5 *Water uptake and water loss by cut spike*:

It is very clear from the data presented in table-30 that water uptake/spike/day was significantly increased by GA_3 application at any of three methods viz. Pre-soaking, foliar and pre-soaking + foliar in comparison to the control (7.00ml). However, combined application of GA_3 (pre-soaking + foliar) proved the most effective in enhancing water uptake (12.90ml). In contrast, application of kinetin either as pre-soaking or foliar or a combination of this two significantly reduced water uptake of the spike in comparison to the control and higher rates of kinetin proved inhibitory to this.

The data recorded for water loss per spike per day is given in table-30. It is obvious from the data that application of both the plant growth regulator at any of the three methods significantly reduced the water loss from the spike in comparison to the control, which recorded the maximum water loss (8.05ml/spike/day). However, minimum water loss (3.80ml/spike/day) was noticed in case of T_4, which was significantly lower than all other treatments except T_5 (4.05ml) and T_8 (3.92ml), where it was at

Table-30 **Mean performance of water relation of spike of different plant growth regulators treatment in *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	Water Uptake (ml/spike/day) 1999-2000	2000-2001	Water Loss (ml/spike/day) 1999-2000	2000-2001
T_1	7.00	6.98	8.05	8.01
T_2	9.28	9.04	4.85	4.92
T_3	9.00	9.12	4.40	4.76
T_4	4.68	4.50	3.80	3.68
T_5	5.22	5.35	4.05	4.18
T_6	9.50	9.05	4.97	4.90
T_7	9.75	9.62	5.03	5.11
T_8	5.20	5.41	3.92	3.90
T_9	6.06	6.10	4.16	4.25
T_{10}	12.90	12.96	5.32	5.41
T_{11}	11.18	11.05	5.29	5.52
T_{12}	5.03	5.17	4.91	4.87
T_{13}	5.85	5.76	5.45	5.56
CD (0.05)	**0.33**	**0.46**	**0.27**	**0.34**

par. In general, kinetin application was more effective in reducing water loss than the GA_3.

The data followed a similar trend during the second year of investigation.

4.12.6 *Time required for opening of basal floret:*

Data recorded for time required for opening of basal floret is presented in table-31. It is clear from the data that time required for opening of basal floret was significantly increased by all the treatments in comparison to the control (2.07day). However, maximum time required for opening of basal floret (5.95 days) was observed under T_{10}, which was immediately followed by T_{11} and T_6. Lower doses of both the plant growth regulators were more effective than higher concentration. In general, GA_3 was much more effective than kinetin. Similar results were obtained during the second year of investigation.

4.12.7 *Number of days to senescence of basal floret:*

Number of days to senescence of basal floret (table-31) were significantly increased by all the treatments over the control (6.48 days), which recorded the earliest senescence of basal floret. However, maximum number of days required for opening of basal floret were noticed under T_9 (10.19 days), which was significantly higher than all other treatments except T_{12} and T_{13}. Pre-soaking of GA_3 was superior to kinetin but in general foliar and pre-soaking + foliar kinetin proved more effective than the GA_3.

Data followed a similar trend during the subsequent year.

4.12.8 *Number of florets/spike open at the time of senescence of basal floret:*

A critical examination of the data (table-31) revealed that there was a significant increase in the number of opened florets/spike at the time of senescence of basal floret due to all the treatments, when compared with the

Table-31 **Mean performance of spike quality of different plant growth regulator treatments in *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	Time required for opening of basal floret(Days)		No. of days to senescence of basal floret		No. of florets/spike open at the time of senescence of basal floret		No. of floret/spike open before stem collapse		Longevity	
	1999-2000	2000-2001	99-00	00-01	99-00	00-01	99-00	00-01	1999-2000	2000-2001
T_1	2.07	2.10	6.48	6.12	4.73	4.42	4.58	4.32	12.48	12.09
T_2	3.13	3.21	8.22	8.32	5.30	5.57	6.19	6.30	15.28	15.36
T_3	3.07	3.09	8.30	8.57	5.29	5.52	5.64	5.82	15.17	14.69
T_4	2.47	2.90	7.75	7.93	5.11	5.15	5.15	5.08	13.58	13.18
T_5	4.25	4.42	8.00	8.10	4.69	4.97	5.02	5.00	13.11	12.12
T_6	5.07	5.04	9.58	9.31	7.23	7.31	7.18	7.15	16.27	16.42
T_7	4.16	4.35	9.71	9.97	7.19	7.14	6.92	6.89	16.25	16.17
T_8	3.25	3.10	9.12	9.17	5.20	5.38	5.75	5.93	14.78	14.21
T_9	3.12	3.19	10.19	10.22	5.15	5.21	5.18	5.25	14.14	13.94
T_{10}	5.95	6.05	9.14	9.11	8.10	7.97	8.11	8.15	16.40	17.00
T_{11}	5.23	5.38	9.10	9.19	7.30	7.45	7.50	7.95	16.27	16.76
T_{12}	3.88	3.98	10.11	10.15	7.05	7.10	6.21	6.34	16.03	16.10
T_{13}	3.22	3.32	10.00	10.16	6.65	6.74	6.17	6.31	15.35	15.52
CD (0.05)	**0.15**	**0.17**	**0.32**	**0.36**	**0.27**	**0.34**	**0.30**	**0.40**	**1.41**	**1.64**

control (4.73). Maximum opened floret (8.10) per spike were observed under T_{10}, which was significantly higher than all the treatments. Lower orates of both the plant growth regulators were more effective than the higher rates. In general efficacy of GA_3 was considerably higher than kinetin, when applied at any of the three methods.

Almost similar observation in the second year of the investigation was noticed.

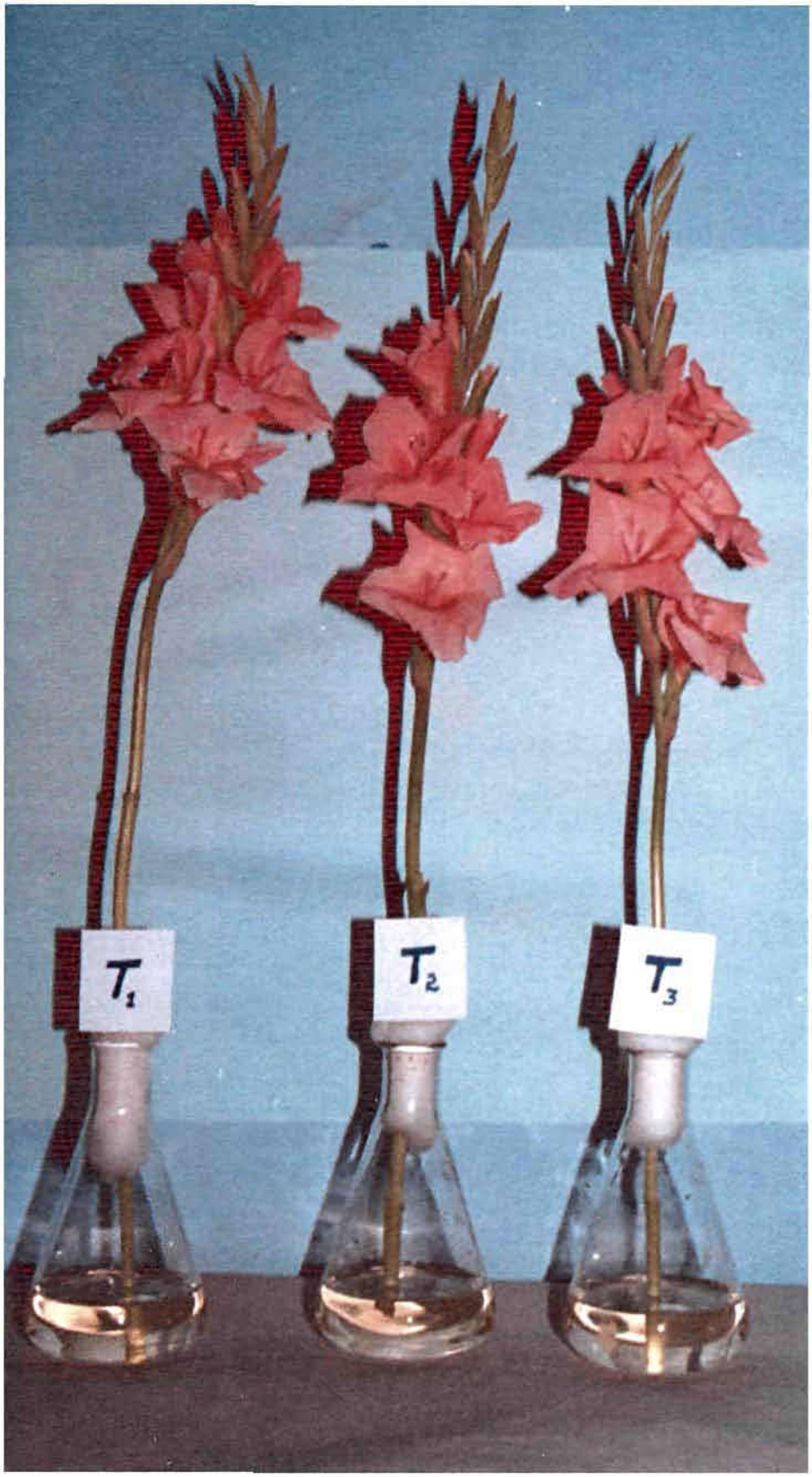
4.12.9 *Number of florets/spike open before wilting or stem collapse*:

It is further clear from the data given in table-31 that number of opened florets before stem collapse were significantly increased by both the plant growth regulators at both the concentration and at all the three methods of application as compared to the control (4.58). However, GA_3 proved more effective than kinetin irrespective of the method of application. Higher concentration did not produce additional gain over their lower rates. Maximum opened florets per spike were recorded under T_{10}, which was significant by higher than all other treatments and was immediately followed by T_{11} (7.50) and T_6 (7.18). Similar observations were recorded during second year of investigation.

4.12.10 *Longevity*:

Data recorded on the longevity of cut spike is given in table-31. It is evident from the data that longevity of cut spikes was significantly increased by all the treatments in comparison to the control (12.48 days) except T_4 (13.58 days) and T_5 (13.11 days). However, maximum flower longevity (16.40 days) was observed under T_{10}, which was at par with T_2, T_3, T_6, T_7, T_{11}, T_{12}, and T_{13}. Higher concentration of either of the two plant growth regulators at any of the three methods of application did not give better results over the lower concentration.

Almost similar observation in the second year of the investigation was noticed.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T2
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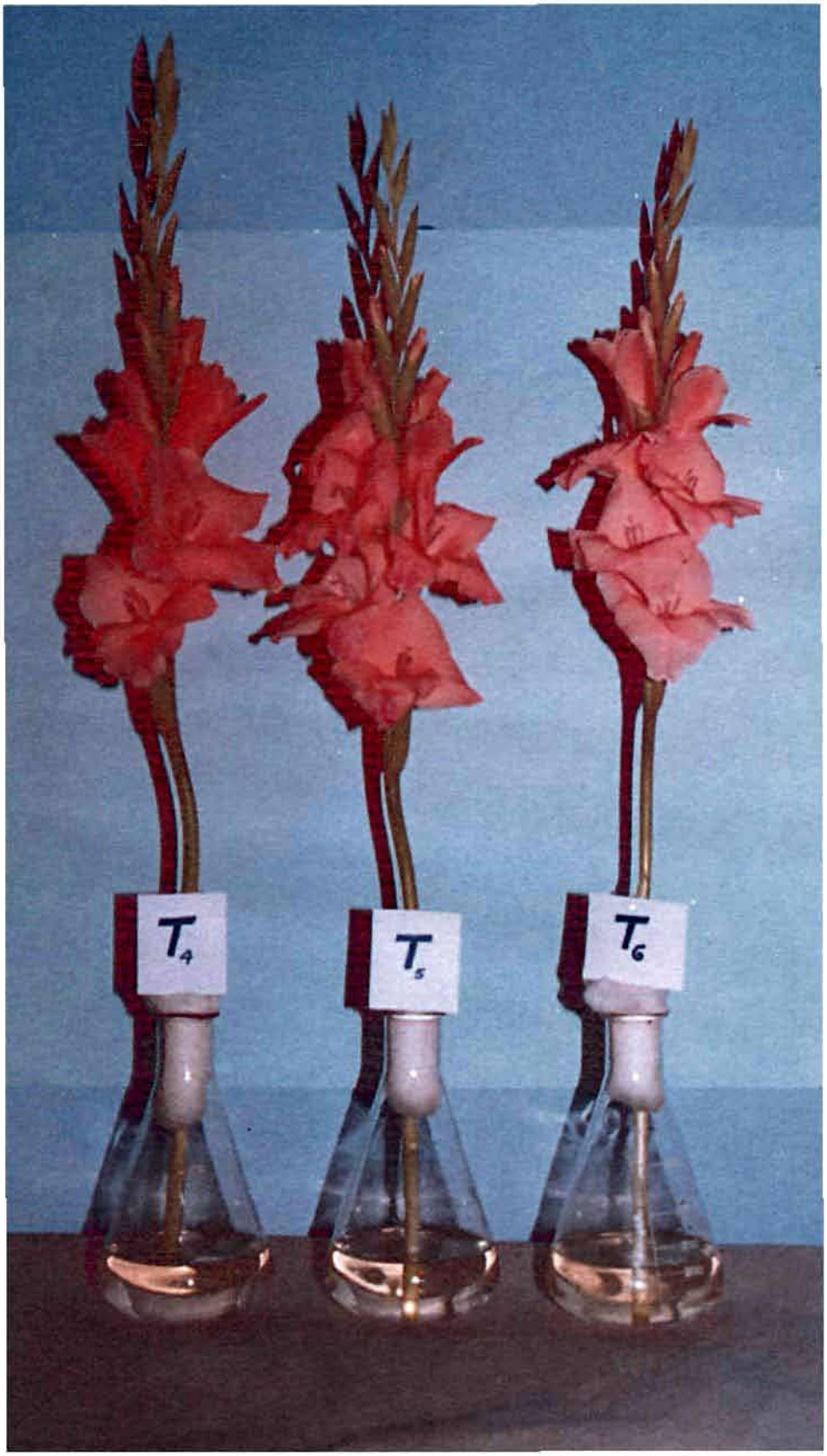
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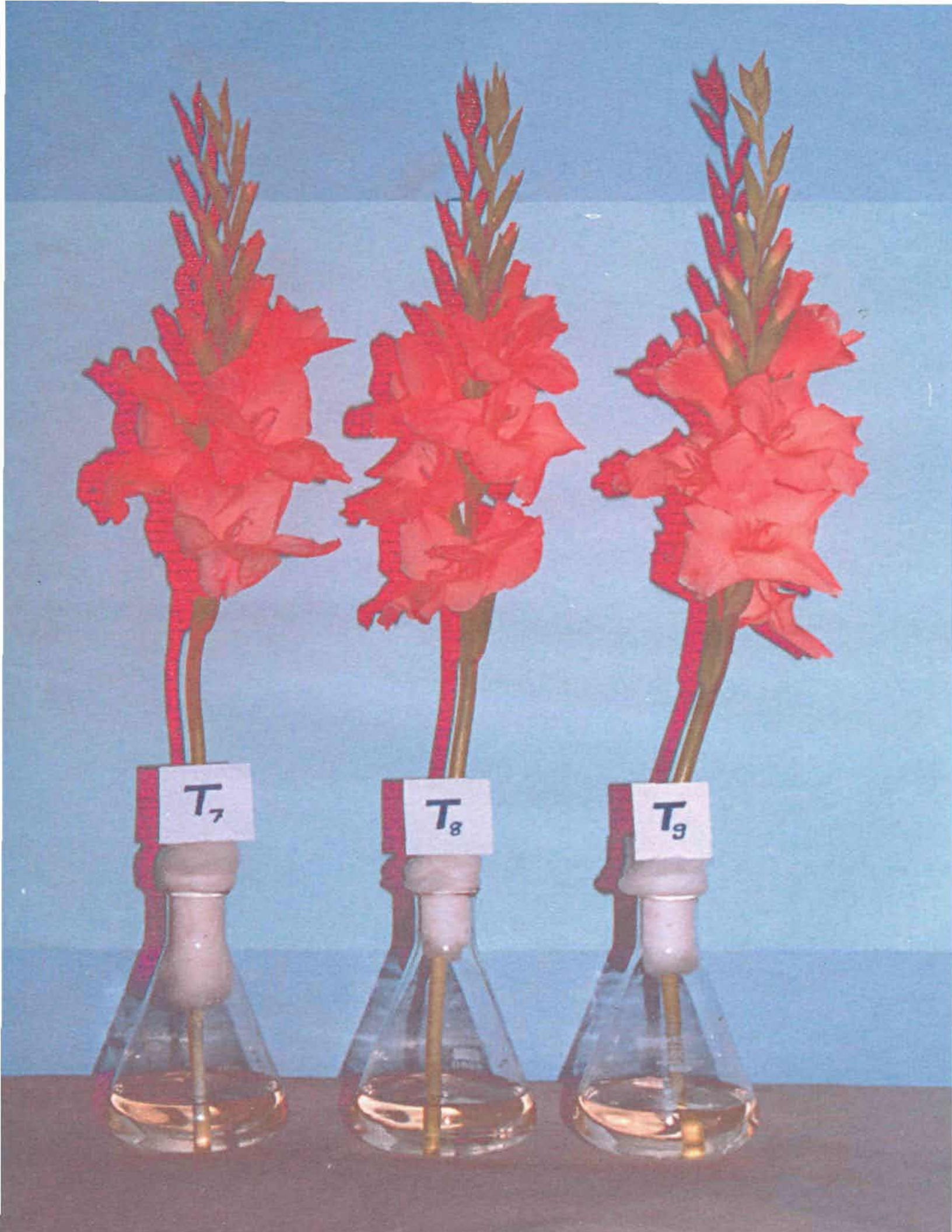
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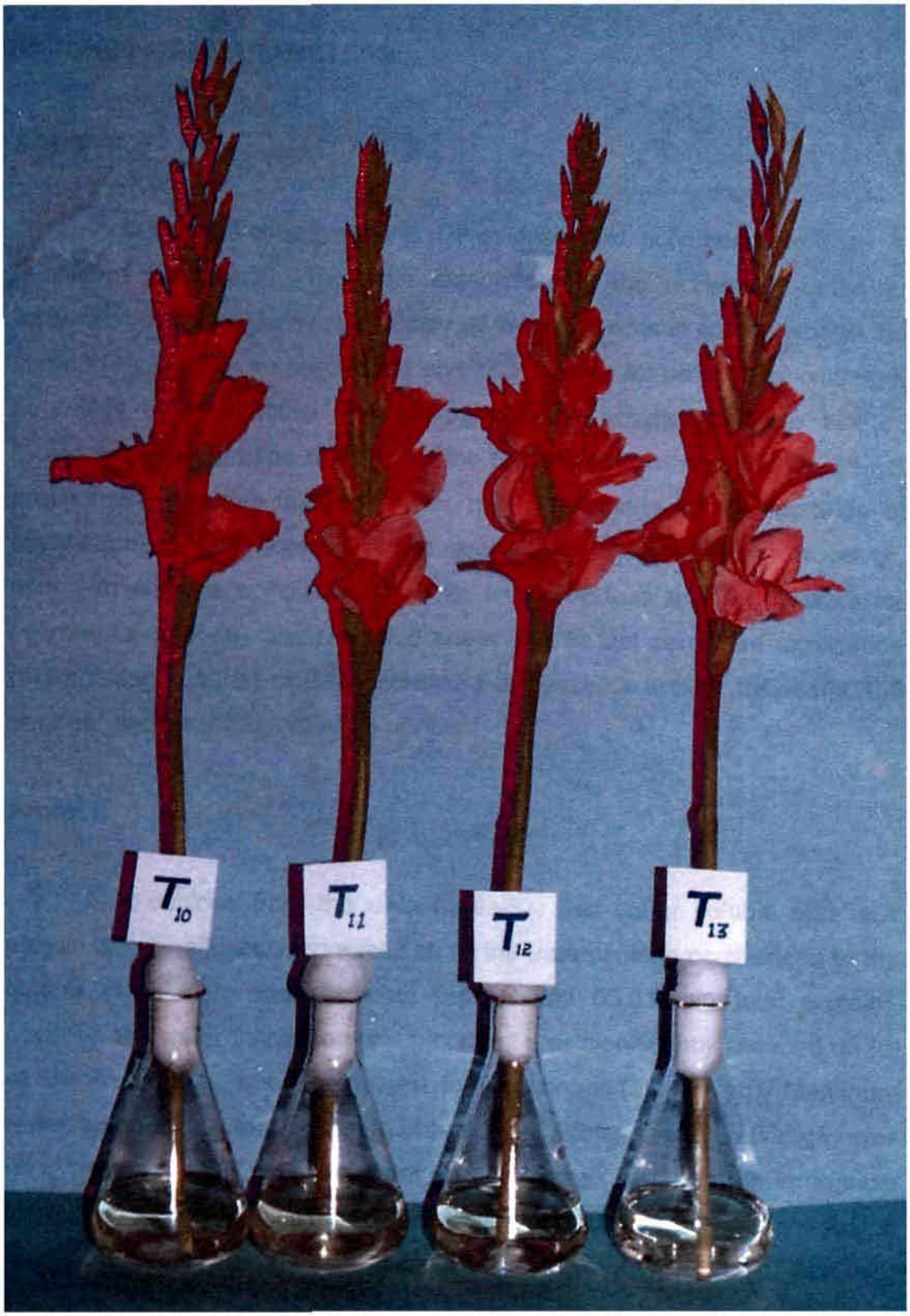
T10
T11
T12
T13

4.13 BIO-CHEMICAL PARAMETERS:

4.13.1 *Enzyme*:

Activity of the enzyme viz. Peroxidase and polyphenol oxidase is presented in table-32. The data exhibited that the activity of both the enzymes was significantly reduced by all the treatments in comparison to the control (89.60 units for peroxidase and 152.64 units for polyphenol oxidase). GA_3 was more effective in reducing enzyme activity than the kinetin. Increased concentration of either of the two plant growth regulators did not prove more effective than the lower rate, irrespective of the methods of application i.e. pre-soaking, foliar and pre-soaking + foliar. However, minimum enzyme activity (32.00 units for peroxidase and 60.16 units for polyphenol oxidase) was recorded under T_{10}. In fact combined application (pre-soaking + foliar) of GA_3 decreased the enzyme activity by nearly 150 per cent and kinetin by 100-125 per cent.

4.13.2 *Protein*:

It is obvious from the data (table-33) that water soluble and total protein was significantly increased by all the treatments in comparison to the control (15.84mg/g water soluble protein and 55.31mg/g total protein), whereas the water insoluble protein content was significantly reduced under all the treatments when compared to the control (39.47mg/g). Maximum water soluble and total protein content of 45.36mg/g and 65.96mg/g was observed under T_{10}, whereas water insoluble protein was maximum in case of control (39.47mg/g). Higher concentration of either of the two plant growth regulators failed to further increase in the water soluble and total protein over the lower concentration. Reduction in water insoluble protein was much more pronounced under GA_3 than the kinetin.

Table-32 **Mean enzyme activity performance of different plant growth regulators treatment in fresh petal of *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	Peroxidase 1999-2000	Polyphenol oxidase 1999-2000
T_1	89.60	152.64
T_2	72.96	121.92
T_3	74.56	125.76
T_4	64.96	86.40
T_5	84.80	143.04
T_6	66.56	95.68
T_7	66.88	101.12
T_8	79.68	139.20
T_9	84.48	139.37
T_{10}	32.00	60.16
T_{11}	48.96	77.76
T_{12}	68.16	114.56
T_{13}	69.76	120.32
CD (0.05)	**13.06**	**12.62**

* Mean enzyme activity have been expressed as units**/gfw/h, except for peroxidase where it is units/gfw/min.

** I Unit = Change in 1.0 O.D for the enzyme peroxidase and Poly phenol oxidase at 430nm, respectively.

Table-33 Mean protein content of different plant growth regulators treatment in fresh petal of *Gladiolus grandiflorus* L. cv. Jessica.

Treatments	Water Soluble Protein (mg/g) 1999-2000	Water Insoluble Protein (mg/g) 1999-2000	Total Protein (mg/g) 1999-2000
T_1	15.84	39.47	55.31
T_2	29.83	30.83	60.66
T_3	29.78	30.94	60.72
T_4	22.09	36.71	58.80
T_5	21.50	37.22	58.72
T_6	39.83	22.69	62.52
T_7	36.72	25.28	62.00
T_8	28.68	32.01	60.69
T_9	26.09	35.32	61.41
T_{10}	45.36	20.60	65.96
T_{11}	44.87	19.16	64.03
T_{12}	32.77	29.22	61.99
T_{13}	30.79	30.42	61.21
C.D (0.05)	1.32	1.39	1.35

4.13.3 *Nucleic acid*:

Data regarding RNA and DNA content of spike is presented in table-34. It can be inferred from the data that all the treatments significantly increased the RNA and DNA content. Higher concentration of either of two plant growth regulators did not produce additional response. However, pre-soaking + foliar application of both the plant growth regulators proved the most effective. In general, efficacy of GA_3 in enhancing the nucleic acid was considerably more than the kinetin. Maximum RNA (200.95μg/g) and DNA (83.04μg/g) content was recorded under T_{10}, which were significantly higher than all other treatments. However, minimum RNA (110.86μg/g) and DNA (42.97μg/g) was observed in the control. Similar results were obtained in the following year.

4.13.4 *Total carbohydrate, starch and phenol*:

Data regarding reducing and non-reducing sugars, total sugars, starch and phenol content are given in the table-35. The data revealed that there was a significant increase in the reducing sugar under all the treatments in comparison to the control (4.59mg/g). However, GA_3 was more effective in increasing reducing sugar particularly when applied as foliar spray or in combination of pre-soaking and foliar spray. Higher concentration of the plant growth regulators proved inhibition to the increase in reducing sugar over their lower rates. Maximum reducing sugar (32.01mg/g) was observed under T_{10}, which was significantly higher than all other treatments and was followed by T_{11} and T_6. In contrast to the changes in reducing sugar, non reducing sugar were significantly reduced by all the treatments in comparison to the control (12.89mg/g), which recorded highest non reducing sugar (table-35). The differences between the concentration of the plant growth regulators irrespective of the methods of application were non-significant. Foliar and particularly the combined application (pre-soaking +

Table-34 **Mean Nucleic acid content of different plant growth regulators treatment in fresh petal of *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	RNA (μg/g) 1999-2000	DNA (μg/g) 1999-2000
T_1	110.86	42.97
T_2	155.62	61.98
T_3	129.60	59.75
T_4	117.49	47.38
T_5	107.86	44.16
T_6	175.48	73.12
T_7	173.77	73.94
T_8	127.76	55.55
T_9	122.41	54.65
T_{10}	200.95	83.04
T_{11}	197.32	78.61
T_{12}	173.24	72.79
T_{13}	156.87	61.28
CD (0.05)	**2.39**	**1.27**

Table-35 **Mean carbohydrate, starch and phenol content of different plant growth regulators treatment in fresh petal of *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	Reducing Sugar (mg/g) 1999-2000	Non-Reducing Sugar (mg/g) 1999-2000	Total Carbohydrate (mg/g) 1999-2000	Starch (mg/g) 1999-2000	Phenol (mg/g) 1999-2000
T_1	4.59	12.89	18.15	1.27	1.89
T_2	16.26	6.07	22.64	2.96	1.34
T_3	14.50	6.45	21.28	2.58	1.40
T_4	9.57	9.11	19.15	2.02	1.61
T_5	7.87	10.51	18.93	1.96	1.74
T_6	24.54	3.69	28.42	3.55	1.05
T_7	19.84	4.97	25.07	3.54	1.17
T_8	13.33	7.19	20.89	2.30	1.43
T_9	10.64	8.66	19.75	2.13	1.53
T_{10}	32.01	1.40	33.48	4.32	0.93
T_{11}	26.40	3.60	30.18	3.78	0.95
T_{12}	17.92	5.56	23.77	3.54	1.19
T_{13}	16.51	5.75	22.56	3.34	1.23
CD (0.05)	**1.42**	**1.38**	**1.41**	**0.32**	**0.13**

foliar) of GA_3 most drastically reduced the non-reducing sugar. Minimum (1.40mg/g) non-reducing sugar was observed under T_{10}.

It is further clear from the data presented in table-35 that total sugar were significantly increased by GA_3 treatment either as pre-soaking, foliar and combination in comparison to the control (18.15mg/g), whereas only foliar and combined application gave the significant results and pre-soaking was not effective of the two plant growth regulators, GA_3 was much more effective particularly the combined application. Increased concentration of the plant growth regulators did not give additional gain. However, maximum total sugar (33.48mg/g) was recorded under T_{10}, which was significantly higher than all other treatments.

All the plant growth regulator treatments increased the starch content as compared to the control (1.27mg/g) which recorded the lowest value. Increment in starch content was more pronounced with GA_3 particularly when applied in combination. Kinetin treatment generally proved inhibitory to increase in starch. Maximum (4.32mg/g) was observed under T_{10}. Data exhibited nearly same trend in the following year also.

A perusal of data (table-35) also showed that there was a significant decrease in phenol content under all the treatment when compared with control (1.89mg/g). However, minimum phenol (0.93mg/g) was noticed under T_{10}, which was significantly lesser than all other treatments. Higher concentration of both the plant growth regulators proved acceleration to the increase in phenol. Pre-soaking + foliar application of both the plant growth regulators was the most effective.

4.13.5 *Anthocyanin content*:

The data recorded with respect to pelargonidine, delphinidine, peonidine and total anthocyanin content is given in table-36. A critical evaluation of the data revealed that maximum pigment content viz. 0.72mg/g pelargonidine, 0.77mg/g delphinidine, 0.86mg/g peonidine and 1.72mg/g total anthocyanin was observed under T_{10}. Increase in concentration either of the two plant growth regulators, irrespective of the method of application

Table-36 **Mean anthocyanin content of different plant growth regulators treatment in fresh petal of *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	Pelargonidine (mg/g) 1999-2000	Delphinidine (mg/g) 1999-2000	Peonidine (mg/g) 1999-2000	Total anthocyanin (mg/g) 1999-2000
T_1	0.11	0.14	0.11	0.26
T_2	0.35	0.39	0.48	1.14
T_3	0.35	0.39	0.46	1.09
T_4	0.23	0.27	0.33	0.45
T_5	0.11	0.16	0.26	0.35
T_6	0.65	0.72	0.84	1.67
T_7	0.51	0.59	0.66	1.48
T_8	0.33	0.36	0.41	0.59
T_9	0.31	0.28	0.37	0.59
T_{10}	0.68	0.77	0.86	1.72
T_{11}	0.66	0.77	0.84	1.68
T_{12}	0.36	0.42	0.51	1.27
T_{13}	0.36	0.41	0.51	1.26
CD (0.05)	0.34	0.34	0.31	0.36

did not contribute to the increase over the lower rate. However, combined application (pre-soaking + foliar) of GA_3 proved the most effective in enhancing all the pigment constituents under study.

4.13.6 *Carotenoid content*:

It is obvious from the data (table-37) that α and β- carotene and total carotenoid were significantly increased by all the plant growth regulators in comparison to the control i.e. 0.63mg/g α-carotene, 0.76mg/g β- carotene and 0.73mg/g total carotenoid. Maximum value of α-carotene (2.92mg/g), β-carotene (2.41mg/g) and total carotenoid (2.47mg/g) were recorded in treatment T_6, which were significantly higher than all other treatments. Higher concentration of both the plant growth regulators could not result in additional gain, rather proved inhibitory. Pre-soaking + foliar application was found best, followed by foliar application and pre-soaking was the least effective.

4.14 pH:

Changes in pH as a result of different plant growth regulators are given in table-38. There was a considerable decrease in pH under all the plant growth regulators treatments and this decrease was more pronounced under GA_3 treatment, irrespective of the method adopted. Changes in pH under different concentration of either of the two plant growth regulators were not appreciable. However, maximum reduction in pH (3.65 and 3.45 during 1999-2000 and 2000-2001) under treatment T_{10}. Control recorded the highest pH values in both the years of investigation.

Table-37 Mean carotenoid content of different plant growth regulators treatment in fresh petal of *Gladiolus grandiflorus* L. cv. Jessica.

Treatments	α-Carotene (mg/g) 1999-2000	β-Carotene (mg/g) 1999-2000	Total Carotenoid (mg/g) 1999-2000
T_1	0.63	0.76	0.73
T_2	1.32	1.32	1.34
T_3	1.26	1.26	1.24
T_4	0.99	0.94	1.11
T_5	0.88	0.75	1.09
T_6	2.92	2.41	2.47
T_7	2.38	2.19	2.24
T_8	1.16	1.14	1.19
T_9	1.08	0.98	1.16
T_{10}	1.62	2.02	4.25
T_{11}	1.32	1.55	3.44
T_{12}	1.19	1.16	1.46
T_{13}	1.18	1.16	1.39
CD (0.05)	**0.11**	**0.11**	**0.10**

Table-38 Mean pH of different plant growth regulators treatment during petal senescence in *Gladiolus grandiflorus* L. cv. Jessica.

Treatments	Changes in pH 1999-2000	2000-2001
T_1	4.10	4.25
T_2	3.97	3.85
T_3	3.97	3.95
T_4	4.02	4.05
T_5	4.03	4.10
T_6	3.90	3.80
T_7	3.91	3.85
T_8	4.00	4.00
T_9	4.02	4.00
T_{10}	3.65	3.45
T_{11}	3.88	3.55
T_{12}	3.92	3.95
T_{13}	3.92	3.95
CD (0.05)	**0.15**	**0.19**

Initial pH : 3.5

4.15 Ethylene evolution:

Data pertaining to table-40 reveal that the rate of evolution of ethylene was significantly reduced by all the treatments as compared to the control i.e. 1.67nl/g/fw/hr, 3.28nl/g/fw/hr and 2.65nl/g/fw/hr on phase-1, phase-2 and phase-3, respectively except under T_4 and T_5 which were at par with control. GA_3 application was more effective in reducing the evolution of ethylene than kinetin. However, combined application (pre-soaking + foliar) of GA_3 100ppm i.e. T_{10} (0.82 nl/g/fw/hr, 1.08 nl/g/fw/hr and 1.02 nl/g/fw/hron phase-1, phase-2 and phase-3 was the most effective. There were no significant differences between the lower and higher doses of either of the two plant growth regulators, irrespective of the phase of observation. It is further clear from the data that the rate of evolution of ethylene increased on phase-2 in comparison to the phase-1, with a subsequent decline regardless of the treatments.

4.16 Respiration:

The data regarding the rate of respiration of the floret is given in table-41. It is evident from the data that the rate of respiration was significantly reduced by all the plant growth regulators treatment in comparison to the control, which recorded 486.64µl/gfw/hr, 938.55µl/gfw/hr and 799.88µl/gfw/hr of CO_2 on phase-1, phase-2 and phase-3, respectively. Lower concentration of both the plant growth regulators were more effective than the higher rates in this regard. However, pre-soaking + foliar GA_3 100ppm (T_{10}) was the most effective in reducing the rate of respiration, which produced 217.49µl/gfw/hr, 552.57µl/gfw/hr and 403.27µl/gfw/hr of CO_2 in phase-1, phase-2 and phase-3, respectively. Highest rate of respiration was observed under control (T_1) regardless of the phase of observation.

Table-40 Mean ethylene production (nl/gfw/hr) of different plant growth regulators treatment in fresh petal of *Gladiolus grandiflorus* L. cv. Jessica.

Treatments	Phase 1 1999-2000	Phase 2 1999-2000	Phase 3 1999-2000
T_1	1.67	3.28	2.65
T_2	1.42	2.40	1.96
T_3	1.47	2.59	2.07
T_4	1.58	2.90	2.49
T_5	1.59	3.04	2.50
T_6	1.07	1.65	1.18
T_7	1.08	1.82	1.24
T_8	1.52	2.85	2.13
T_9	1.53	2.89	2.30
T_{10}	0.82	1.08	1.02
T_{11}	1.03	1.61	1.07
T_{12}	1.11	1.86	1.24
T_{13}	1.18	1.90	1.40
CD (0.05)	0.11	0.13	0.19

Table-41 Mean respiration production (µl/gfw/hr) of different plant growth regulators treatment in fresh petal of *Gladiolus grandiflorus* L. cv. Jessica.

Treatments	Phase 1 1999-2000	Phase 2 1999-2000	Phase 3 1999-2000
T_1	486.64	938.55	799.88
T_2	306.21	724.01	612.14
T_3	377.14	742.67	631.56
T_4	399.35	796.33	666.83
T_5	435.26	879.34	676.01
T_6	247.29	630.32	536.58
T_7	264.97	697.77	544.55
T_8	377.57	752.81	645.72
T_9	386.24	777.61	663.13
T_{10}	217.49	552.51	403.27
T_{11}	233.84	625.16	492.52
T_{12}	265.34	719.97	570.07
T_{13}	295.13	723.86	609.81
CD (0.05)	**6.89**	**5.74**	**8.92**

4.17 SENESCENCE PARAMETERS:

4.17.1 *Protein content*:

The data regarding water soluble, water insoluble and total protein during petal senescence are presented in table-42. It is apparent from data that all the plant growth regulators as compared with control (31.13mg/g) significantly increased water-soluble protein. However, maximum water soluble protein content (51.93mg/g) was recorded under T_{10}, which was significantly higher than all other treatments. In general, application of GA_3 was more effective than the kinetin. It is also clear that higher concentration of either of the two-plant growth regulator did not give additional gain.

Data presented in table-42 further exhibited that there was a significant reduction in water insoluble protein due to all the plant growth regulator treatments during senescence as compared to control (9.89mg/g). However, this reduction was the most pronounced (0.14mg/g) under T_{10}, which was at par with T6 (0.46mg/g) and T_{11} (0.37mg/g). Higher concentration of GA_3 was more effective in reducing the loss of water insoluble protein. Pre-soaking with kinetin (T_4) recorded the maximum water insoluble protein (7.48mg/g) after the control. Higher rates of kinetin at any method of application did not produce the additional effect over the lower rate.

A critical examination of data in table-42 also showed that the total protein content was significantly increased by all the plant growth regulators treatment and T_{10} (52.07mg/g) was the most effective in this respect. Application of GA_3 was more effective than the kinetin in enhancing the total proteins, irrespective of the method of application. However, minimum total protein content (41.02mg/g) was noticed under T_1 which was significantly lesser than all other treatments.

Table-42 Mean protein content of different plant growth regulators treatment during petal senescence in *Gladiolus grandiflorus* L. cv. Jessica.

Treatments	Water Soluble Protein (mg/g) 1999-2000	Water Insoluble Protein (mg/g) 1999-2000	Total Protein (mg/g) 1999-2000
T_1	31.13	9.89	41.02
T_2	42.50	1.63	44.13
T_3	41.30	2.38	43.68
T_4	38.91	7.48	46.39
T_5	35.46	7.35	42.81
T_6	46.39	0.46	46.85
T_7	45.49	0.55	46.04
T_8	40.25	4.43	44.68
T_9	40.11	4.32	44.43
T_{10}	51.93	0.14	52.07
T_{11}	49.08	0.37	49.45
T_{12}	44.15	1.04	45.19
T_{13}	42.95	1.32	44.27
CD (0.05)	**0.74**	**0.32**	**0.57**

4.17.2 *Nucleic acid content*:

The data recorded on nucleic acid content at senescence is presented in table-43. It is obvious from the data that the RNA and DNA content of the senescing floret was significantly increased by all the Plant growth regulator treatment in comparison to the control (114.27μg/g and 45.65μg/g), all the plant growth regulator treatment significantly increased the RNA and DNA content of the floret. Combined application of GA_3 (T_{10}) was the most effective (231.45μg/g and 96.44μg/g) in enhancing the RNA and DNA content of the senescing floret. Lower concentration of all the plant growth regulator treatment recorded significantly higher RNA and DNA content than the higher rates except under T_6 and T_7.

4.17.3 *Total carbohydrate, starch and phenol content*:

It is quite obvious from the data (table-44) that there were significant differences in reducing sugar, non-reducing sugar and total sugar due to various plant growth regulator treatments. Reducing sugars were significantly increased by all the treatment in comparison to the control (3.23mg/g), while non reducing sugar and total sugar recorded a significant reduction under all the treatments, when compared with control (30.56mg/g non reducing sugar and 35.40mg/g total sugar). Maximum (16.14mg/g) reducing sugar was recorded under T_{10}, which was significantly higher than all other treatments. GA_3 was considerably more effective in enhancing reducing sugar than the kinetin irrespective of the method of application. In contrast, all the plant growth regulator treatments significantly reduced the non-reducing sugar and total sugar and this reduction was most pronounced under T_{10} (1.84mg/g with respect to non-reducing sugar and T_{11} (18.08mg/g) with respect to total sugar. Maximum non-reducing sugar (30.56mg/g) and total sugar (35.40mg/g) were, however, noticed under control. Reduction in non-reducing sugar and total sugar was much higher with the application of GA_3 than the kinetin, irrespective of the method of an application or

Table-43 **Mean Nucleic acid content of different plant growth regulators treatment during petal senescence in *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	RNA (μg/g) 1999-2000	DNA (μg/g) 1999-2000
T_1	114.27	45.65
T_2	172.70	69.92
T_3	170.78	68.20
T_4	146.38	59.99
T_5	121.02	50.42
T_6	193.25	84.02
T_7	193.25	83.29
T_8	163.39	73.59
T_9	158.90	72.88
T_{10}	231.45	96.44
T_{11}	217.65	88.48
T_{12}	187.79	80.60
T_{13}	173.66	69.19
CD (0.05)	**1.64**	**1.16**

Table-44 Mean carbohydrate, starch and phenol content of different plant growth regulators treatment during petal senescence in *Gladiolus grandiflorus* L. cv. Jessica.

Treatments	Reducing sugar (mg/g) 1999-2000	Non-Reducing sugar (mg/g) 1999-2000	Total Carbohydrate (mg/g) 1999-2000	Starch (mg/g) 1999-2000	Phenol (mg/g) 1999-2000
T_1	3.23	30.56	35.40	0.65	2.59
T_2	11.00	14.16	25.91	0.99	1.86
T_3	11.52	15.36	26.36	0.94	1.92
T_4	8.03	22.29	31.49	0.81	2.32
T_5	6.91	24.85	33.07	0.74	2.38
T_6	13.49	7.04	20.90	1.23	1.41
T_7	12.93	8.43	21.80	1.16	1.67
T_8	10.04	16.52	27.43	0.94	2.02
T_9	8.19	20.05	29.30	0.89	2.14
T_{10}	16.14	1.84	18.08	1.39	1.21
T_{11}	13.49	4.34	18.06	1.29	1.29
T_{12}	12.12	8.95	21.54	1.09	1.67
T_{13}	11.01	10.31	21.85	1.06	1.72
CD (0.05)	**1.32**	**1.54**	**1.15**	**0.18**	**0.38**

concentration. However, the magnitude of reduction was considerably higher with higher concentration of both the plant growth regulators.

Starch content of the senescing floret was significantly increased by all the plant growth regulator treatments in comparison to the control (0.65mg/g). However, increase in starch was most pronounced under T_{10} (1.39mg/g), that was significant higher than all the treatments. Effect of GA3 on increase in starch was much more severe than the kinetin irrespective of the concentration and the method of application (table-44).

It is also clear from the data (table-44) that there was a significant decrease in phenol content due to all the plant growth regulator treatments except T_4 (2.32mg/g) and T_5 (2.38mg/g), in comparison to the control (2.59mg/g). Application of GA_3 was more effective than the kinetin, in the regard, irrespective of the method of application. However, minimum phenol content (1.21mg/g) was observed under T_{10}, which was significantly lesser than all other treatments. Higher concentration of either of the two plant growth regulators did not produce additional response.

4.17.4 *Anthocyanin content*:

The data regarding anthocyanin constituent and total anthocyanin content of senescent floret are presented in table-45. There was a considerable increase in anthocyanin costuents under all the plant growth regulators in comparison to the control (0.29mg/g pelargonidine, 0.33mg/gdelphinidine and 0.42mg/g peonidine, respectively but the differences were significant under T_2, T_3, T_6, T_7, T_{10}, T_{11}, T_{12} and T_{13} only. However, total anthocyanin content was significantly increased by all the plant growth regulator treatments except T_4, T_5, T_8 and T_9. Combined application (pre-soaking + foliar spray) of GA_3 T_{10} proved most effective in enhancing the pelargonidine (0.72mg/g), delphinidine (0.78g/g), peonidine (0.74mg/g) and total anthocyanin (1.18mg/g). None of the pre-soaking treatments and foliar spray of GA_3 could prove effective. However, lower concentration of both the chemicals proved most effective in increasing the

Table-45 **Mean anthocyanin content of different plant growth regulators treatment during petal senescence in *Gladiolus grandiflorus* L. cv. Jessica.**

Treatments	Pelargonidine (mg/g) 1999-2000	Delphinidine (mg/g) 1999-2000	Peonidine (mg/g) 1999-2000	Total anthocyanin (mg/g) 1999-2000
T_1	0.29	0.33	0.42	0.62
T_2	0.44	0.48	0.61	0.85
T_3	0.43	0.46	0.60	0.82
T_4	0.38	0.40	0.56	0.71
T_5	0.36	0.37	0.47	0.63
T_6	0.49	0.54	0.68	1.00
T_7	0.47	0.54	0.66	0.97
T_8	0.42	0.46	0.59	0.80
T_9	0.38	0.42	0.57	0.73
T_{10}	0.72	0.78	0.74	1.18
T_{11}	0.49	0.54	0.72	1.06
T_{12}	0.47	0.54	0.63	0.91
T_{13}	0.46	0.54	0.63	0.85
CD (0.05)	**0.16**	**0.21**	**0.18**	**0.22**

total anthocyanin, irrespective of the method of application and the increase in concentration did not produce further gain.

4.17.5 *Carotenoid content*:

A critical evaluation of the data presented in table-46 revealed that α and β-carotene content was considerably increased by all the treatments in comparison to the control (0.47mg/g and 0.19mg/g), and the differences were significant in all the treatments except T_5. Combined application (pre-soaking + foliar spray) of GA_3 i.e. T_{10} was found most effective in enhancing the α-carotene (1.10mg/g) and β-carotene (1.02mg/g) which was at par with T_{11}, T_{12} and T_{13} for α-carotene and T_{11} for β-carotene. Total carotenoid content was significantly increased by all the plant growth regulator treatments in comparison to the control (0.68mg/g). However, combination of pre-soaking and foliar spray of GA_3 was most effective (1.11mg/g), which was at par with T_6 (1.07mg/g), T_7 (1.02mg/g) and T_{11} (1.11mg/g). Higher concentration of either of the two plant growth regulators did not give additional gain. It was also observed that foliar application of kinetin produced the result comparable to pre-soaking + foliar spray of GA_3.

4.18 CORM PRODUCTION:

Data regarding number of corms and cormels, weight of corm and cormels and diameter of corm are given in table-39. It is evident from the data that there was a significant increase in the number of corms under various plant growth regulators in comparison to the control (4.06) except T_4 (4.30), T_5 (4.25), T_8 (4.19) and T_9 (4.19). However, maximum number of corms was observed under T_{10} (4.83). Higher concentration of either of the two plant growth regulators did not produce additional gain. In general GA_3 was more effective in increasing the corms number, particularly under pre-soaking + foliar application.

Table-46 Mean carotenoid content of different plant growth regulators treatment during petal senescence in *Gladiolus grandiflorus* L. cv. Jessica.

Treatments	α-Carotene (mg/g) 1999-2000	β-Carotene (mg/g) 1999-2000	Total Carotenoid (mg/g) 1999-2000
T_1	0.47	0.19	0.68
T_2	0.87	0.84	0.93
T_3	0.83	0.83	0.90
T_4	0.75	0.78	0.85
T_5	0.64	0.33	0.82
T_6	1.06	0.98	1.07
T_7	0.99	0.94	1.02
T_8	0.83	0.83	0.85
T_9	0.75	0.82	0.85
T_{10}	1.10	1.02	1.11
T_{11}	1.06	1.02	1.11
T_{12}	0.95	0.90	0.98
T_{13}	0.95	0.86	0.93
CD (0.05)	0.26	0.12	0.09

Table-39 Mean performance of corms of different plant growth regulators treatment in *Gladiolus grandiflorus* cv. Jessica.

Treatments	Number of Corms		Number of Cormels		Mean weight of corms(g)		Mean weight of cormels(g)		Diameter of corms(cm)	
	1999-2000	2000-2001	99-00	00-01	99-00	00-01	99-00	00-01	1999-2000	2000-2001
T_1	4.06	4.10	5.97	6.10	19.85	20.26	14.04	13.10	4.70	4.67
T_2	4.67	4.75	7.75	7.96	26.13	26.18	18.34	17.72	5.15	5.43
T_3	4.65	4.69	7.40	7.88	25.25	26.08	17.98	17.61	5.12	5.20
T_4	4.30	4.19	7.11	7.15	24.33	23.50	16.69	16.40	4.85	4.97
T_5	4.25	4.17	7.02	7.04	22.89	23.17	16.06	15.82	4.87	4.92
T_6	4.48	4.28	7.21	7.32	24.49	24.67	17.05	16.88	5.00	5.00
T_7	4.42	4.25	7.21	7.19	24.39	25.16	17.01	16.65	4.92	5.01
T_8	4.19	4.15	6.81	6.97	22.59	23.00	15.71	14.93	4.80	4.89
T_9	4.19	4.13	6.79	6.95	23.28	23.00	15.82	15.25	4.74	4.69
T_{10}	4.83	4.98	8.04	8.20	27.05	27.25	18.77	18.62	5.25	5.95
T_{11}	4.75	4.87	7.94	7.96	26.82	26.48	18.73	18.83	5.22	5.45
T_{12}	4.64	4.51	7.36	7.70	25.16	25.94	17.10	17.49	5.11	5.16
T_{13}	4.55	4.36	7.25	7.38	25.10	25.82	17.49	17.51	5.09	5.09
CD (0.05)	**0.25**	**0.36**	**0.68**	**0.60**	**1.05**	**1.71**	**1.26**	**1.68**	**0.32**	**0.44**

Mean weight of the corm was significantly increased by all the treatments as compared to the control (19.85g). Mean weight of corm (27.05g) was maximum under T_{10}, which was at par with T_{11}. Differences between lower and higher concentration of the plant growth regulator were not significant. Similar results were observed during the second year of investigation. It is also clear from the data (table-39) that the number and weight of the cormels was significant by different plant growth regulators. Maximum number (8.40) and weight (18.77G) of the cormels was observed under T_{10}, which was at par with T_{11}. However, minimum number of (5.97) and weight (14.04g) of cormels was produced by control. Higher concentration of both the plant growth regulators failed to give additional response. In general GA_3 was more effective in enhancing number and weight of the cormels, in comparison to kinetin, particularly when applied in combination (pre-soaking + foliar).

Diameter of the corms was improved by all the treatments over control (4.70cm) but the differences were significant only under T_2, T_3, T_{11}, T_{12} and T_{13}. Maximum corm diameter (5.25cm) was produced by T_{10}. Kinetin applied either, as pre-soaking or foliar application did not yield any response. However, combined application (pre-soaking + foliar) was effective in improving corm diameter. GA_3 application either as pre-soaking, foliar or in combination significantly enhanced the diameter of corm. Higher doses of plant growth regulator did not prove beneficial in comparison to the lower concentration.

DISCUSSION...

The present investigation was undertaken to study the effects of either plant growth regulators (GA_3 and kinetin) in association with VAM or alone as three ways (pre-soaking, foliar spray and pre-soaking + foliar spray) on growth, flowering, physiological and bio-chemical changes during vase-life and corm yield. The results obtained from these investigations, described in the preceding chapter are being discussed in the present chapter. Attempts have also been made to corroborate the findings of present investigation with those of various workers in the past.

Experiment 1 : Studies on the effect of VAM and growth regulators on *Gladiolus grandiflorus* L. cv. Jessica.

5.1 PLANT GROWTH CHARACTERS:

The perusal of data (table-1) on number of days required for sprouting of corms and percentage of sprouting at 30 days after planting revealed that both the plant growth regulators in combination with V.A.M treatments significantly reduced the number of days required for sprouting of corms and were observed minimum at T_{10} (pre-soaking + foliar spray GA_3 100ppm). Significant increase in the percentage of sprouting at 30 days after planting were also noticed with both the plant growth regulators in association with V.A.M treatments and maximum percentage of sprouting was found under T_{10}. These results are in conformity with the findings of Wang *et al.* 1993 and Johnson and Crews (1979). Less number of days required for sprouting of corms and increased percentage of sprouting at 30 days after planting might be due to the symbiotic relationship of VAM with plant host and release cytokinin and gibberellins produced by mycorrhizal fungi (Slankis, 1975; Miller, 1971). It is well documented, the role that cytokinin may play in the mycorrhizal association and for that matter, the role that fungus produced

cytokinin play in sprouting of corms and overall plant growth and development.

5.2 PLANT HEIGHT AND LEAF AREA:

The result of the preceding chapter indicate that the effect of different treatments were found significant on various growth characters at all the stages of crop growth with respect to the plant and leaf area except number of leaves per plant during both the years of trial (table-2).

The maximum plant height were obtained with T_{12} (pre-saoking +foliar spray kinetin 25ppm) in association with V.A.M at all the stages of observation during 1999-2000 and 2000-2001. The increased plant height associated with T_{12} might be due to higher levels of mycorrizal colonization shows growth enhancement resulting from the symbiotic relationship with the plant. Baylis (1972) proposed 'root hair hypothesis' that plants are most likely to respond to V.A.M fungi if their root anatomy allows infection and showed that secondary roots and roots with root hair are more sensitive to V.A.M infection than tap root and also observed colonization in gerbera and nephrolepis more since it have well developed root systems with abundant root hairs like gladiolus root hair. Increased growth of inoculated plants could have been caused by neutralization of soil toxicity by microorganism.

Like wise the maximum number of leaves were obtained with T_{12}, since symbiotic relationship of plant with V.A.M fungi stimulates the growth and development of the plants (Biermann and Lindermann, 1983). V.A.M fungi also responsible for increasing the uniformity and reduces mortality because of better absorption of plant immobile element (Ames *et al.*, 1986) and perhaps the fungus takes over cytokinin synthesis or high indigenous concentration in the root, modifies growth and predisposes the root to mycorrhizal infection (Crafts and Miller, 1974).

Increased leaf area was associated with T_{12} may be due to the increased photosynthetic activity as a result of higher light intensity. Since, mycorrhizal development is greatest at higher light intensity (Bjorkman, 1970). V.A.M are also capable of increasing P_4 uptake and over all plant

growth responses are often the result of increased P_4 nutrition (Mosse, 1973).

Thus, by its very function T_{12} might have improved the vegetative growth characters of gladiolus are in line with the reports of the various workers, Crews *et al.*, 1978; Vanderploeg *et al.*, 1972; Soroa *et al.*, 1998; Bush and Lelly, 1997 and Ames and Lidermann, 1978. Maximum plant height, leaf area associated with T_{12} may also be due to the increase in mineral uptake as the result of mycorrhizal association reflected in increasing plant survival, growth and yield. Increased plant height associated with T_{12} may also be due to the mycelial growth of symbiotic fungi regulated by cytokinin and unidentified substance termed M-factor stimulated mycelial growth of symbiotic fungi (Melin, 1963) and these M-factor may be related to cytokinin (Gogala, 1970).

5.3 FLOWERING CHARACTERS:

The perusal of data on flowering characters is presented in table-3 and 4. Flowering behavior of gladiolus were also influenced markedly by GA_3 and kinetin in association with V.A.M treatment. The minimum number of days required for spike initiation, maximum number of florets/spike and fresh weight of spike at the time of harvesting were observed with T_{12} followed by T_3, T_4, T_5, T_{10}, and T_{11} which were significantly superior to control (T_1). All these attributes influenced perhaps due to the better nutrient absorption during reproductive phase, which resulted in, increased rate of photosynthesis and ultimately showed beneficial effect. Since, the fungal symbiont must enter and maintain a parasitic relationship with its host for procurement of organic compound required for its growth. Meyer (1974) suggested that the fungus intervene in the carbohydrate metabolism of the host by acting as a sink. Whereas, Ho and Trappe (1975) reported that endomycorrizal root of onion contained more total lipid. The abundance of lipid globules could be the result of the fungus acting as an alternative sink for photosynthate. These results corroborate with the finding of Chen and

Chang, 1996; Wang *et al.*, 1993; Daft and Okusanya, 1973; Aboul-Nasr, 1994 and Bagyaraj and Powell, 1985.

Phosphorus is responsible for earliness in flowering and V.A.M are capable of increase in P_4 uptake. Therefore, minimum number of days required for spike initiation was related with T_{12}. The available P_4 transported by the V.A.M to the plant via cytoplasmic streaming occurs with the P_4 being contained in the vacuoles (Cox *et al.*, 1975). Whereass, Bower (1975) suggested that increased nutrient uptake through mycelial strands of fungus could be attributed to solubilization of elemental compound, use of one or more elemental source; rate of translocation to the plant, increased root surface to volume ratio and permeation by hyphal strands into soil regions inaccessible by root hairs.

5.4 PHYSIOLOGICAL CHANGES DURING VASE-LIFE:

The data recorded on physiological changes during vase-life are depicted in table-5, 6, 7 and 8. Daily elongation of cut spike, days to elongation of cut spike, length and diameter of floret, water uptake and loss and quality parameters of spike were influenced by lower concentration of plant growth regulators in association with V.A.M when applied as a three method (pre-soaking, foliar and pre-soaking + foliar). All these characters were showed increased response which are associated with T_{12} (pre-soaking + foliar spray kinetin 25ppm) except water loss was minimum under T_{12} which are prerequisite with the treatment.

Increased elongation of spike associated with T_{12} may be attributed due to the more water uptake which may help in cell elongation by maintaining turgidity while keeping in standard vase solution containing sucrose and 8-HQC but increased rate of elongation was observed over a certain period after that elongation was sustained might be due to high water potential maintained within the xylem vessels of cut spike or cells attain maximum enlargement. There may also be the possibility of occlusion of microbial origin as a gummy substance, pertinacious or carbohydrate in nature.

Length and diameter of flower might have enhanced due to increase in the length of petals and pedicel which is attributable to the drawing of photosynthates to the flower as a consequence of intensification of the sink (Carpenter and Carlson, 1970 and Zeislin *et al.*, 1974). While decreased floret size associated with T_1 (control) may be due to a decreased rate of water uptake (Halevy and Mayak, 1981).

Increased water uptake and minimum water loss was observed under T_{12}. Since, sucrose in holding solution provides an energy source, also act as antidessicant and inclusion of 8-HQC prevent wilting due to an antitranspirant activity (Stoddard and Miller, 1962) and dessication of the flower stems by inhibiting vascular blockage and allowed greater water conductivity (Larson and Cromarty, 1967; Larson and Frolich, 1969) because of reduced bacterial growth in holding solution. Also, the inclusion of germicide like 8-HQC to vase solution partially decreases the resistance to water flow. Generally, the water uptake of freshly cut flowers may initially be high when the plant has a low water potential at cutting. The rate of uptake will reach a steady state corresponding to the rate of transpiration then after subsequently decrease due to the water deficit when amount of transpiration exceeds absorption and this deficit is apparently the resistance to water flow which develop in the stem may be the reason for water loss in Control. Higher rate of transpiration than absorption result in a negative water balance causing decrease in water potential and stomatal closure (De Stigter, 1980a,b).

Quality parameters viz. increase in time required for opening of basal floret, number of days to senescence of basal floret, number of florets/spike open at the time of senescence of basal floret, number of florets/spike before stem collapse and longer vase-life were also associated with T_{12} (pre-soaking + foliar spray kinetin 50ppm) followed by T_{10} (pre-soaking + foliar spray GA_3 100ppm). Enhancement in quality parameters may be due to the improved water balance in flowers throughout the vase-life by closing the stomata and reducing water loss (Marousky, 1971). Increased vase-life with kinetin probably due to the nucleic acid and protein metabolism. Larson and Cromarty (1967) suggested that the extended life of cut flowers might be the result of bacterial properties of quinoline salts, respond to more water

absorption because of reduced bacterial stem blockage. 8-HQC has the chelating properties due to the presence of quinoline salts probably chelated the metal ions of enzyme active in creating the stem blockage and may affect the flower longevity of acidifying the vase solution. Whereas, improvement of floret opening with vase solution containing sucrose + 8-HQC may be due to the accumulation of sugars in the flowers, increased osmotic concentration and maintains the petal turgidity (Halevy, 1976).

5.5 BIO-CHEMICAL CHANGES DURING VASE-LIFE:

It is obvious from the findings obtained through various bio-chemical changes, signifies that to improve the longevity or enhancement of petal senescence various internal as well as external changes occurs, affected mainly due to the metabolic changes by various treatments while keeping in standard vase solution of 8-HQC + sucrose. The data observed by series of bio-chemical changes during the vase-life in table-9, 10, 11, 12, 13 and 14 showed decrease in enzyme activity, increase in water soluble protein, nucleic acid, reducing sugar, total sugars, decrease in starch and phenol and considerable increase in total anthocyanin and carotenoid were under T_{12}.

Decrease in enzymatic activity is the result of the use of flower preservative. Increased enzymatic activity was observed with control (T_1) may be due to the increase in peroxides and free radicals which react with cellular constituents (Fridovich, 1975) and are involved in promotion of senescence (Baker *et al.*, 1977). Stem plugging can be responsible of increased enzymatic activity, which hydrolyses the cell wall material into vessel plugging material (Burdett, 1970). Also, there may be the possibility of free radical which attack on membrane lipids and can be considerred the part of the process leading to leakage of the cell constituents and cell death (Winstein, 1990; Abeles *et al.*, 1992 and Leshem, 1992).

Increased protein content and nucleic acid may be ascribed through specific activities of the protein and RNA, which are maintained in the

presence of kinetin could be shown to an increase in the synthesis of particular fraction of protein and RNA.

The reducing sugar was also noticed higher under T_{12}, since it is predetermined fact that absorbed sucrose in petal are rapidly converted to reducing sugar which then accumulate in the corolla (Wagner, 1979). Whereas, decrease in starch content may be temporarily associated with rapid tissue expansion or increase in fresh weight of the spike and the quantitative disappearance of starch with a corresponding increase in glucose, fructose and to lesser extent sucrose and the starch hydrolysis provides osmotic solutes of water influx and cellular expansion.

During vase-life studies of cut gladiolus spike, the increased synthesis of anthocyanin and carotenoid was noticed and was found that enhanced pigmentation in petal due to higher concentration of pelargonidine with respect to delphinidine and peonidine. Clearly indicates that pelargonidine is the sole constituent of anthocyanin content responsible for the pigmentation in the petal and rapid accumulation of anthocyanin in petal has commonly been observed in the later stage of flower development (Davies *et al.*, 1993) and this rapid increase in anthocyanin is caused by de novo synthesis (Amerhein and Frank, 1989). The induction of anthocyanin synthesis requires the presence of sugar in the medium and sugars may serve as specific signals for the activation of specific genes, as cellular osmotic regulators, or as a general energy source of carbon metabolism in the developing flower (Delila *et al.*, 1997). Whereas, regarding carotenoid content, α-carotene content was maximum than β-carotene content indicates that reddish yellow pigmentation in gladiolus floret was due to the presence of pelargonidine andα-carotene pigment constituent.

5.6 pH :

Data recorded on pH changes over the initial standard value of 3.5 is presented in table-15 reveals that at the termination of vase-life of cut gladiolus, the minimum pH was associated with T_{12} whereas, increased pH

was observed in Control. Low pH throughout the vase-life might prevent bacterial growth, increased water conductivity of stems and inhibits vascular blockage resulted the increased longevity. While, increased pH in control might be a result of NH_3 release due to protein degradation (Weinstein, 1957).

5.7 ETHYLENE AND RESPIRATION:

Since, gladiolus is considered as ethylene insensitive flower (Reid and Wu, 1991) but ethylene plays a major role in senescence of cut spike during the vase-life. The data recorded on ethylene production and respiration are presented in table-17 and 18 reveal that low rate of ethylene production during phase-1, subsequently increased during phase-2 then after sharp decline in phase-3 were influenced by all the treatments. Since, time course of ethylene production follows a typical profile composed of three distinct phases, 1. Slow steady rate 2. An accelerated rises in maximum emanation and 3. A last phase in which production is declining. Thus, emanation might be influenced by changes either in the capacity of ethylene binding sites (Jerie *et al.*, 1978) or ethylene metabolism rate (Beyer, 1978). Minimum ethylene production observed under T_{12}, might be due to reason that cytokinin counteract the ethylene production. An increase in the intercellular concentration in phase-2 leads to an increase in tonoplast permeability (Mayak *et al.*, 1977). Increased ethylene biosynthesis under T_{12} during the phase-2 may also be due to the lack of carbohydrate content and can be promoted due to the autocatalytic action.

Like ethylene, the rate of respiration also followed the same trend and the magnitude of reduction in rate of respiration was highest under T_{12} as compared to the control. The rise in respiration rate at phase-2 may be suggested due to the formation of free radicals with high oxidation potential and this increase in respiration due to the climacteric stage of the flower and may be accompanied by an increase in fresh weight and cell size. Rapid drop in respiration during phase-3 consistent with the poor export of sugars and amino acid. Since, respiration is required for degradation and

metabolization of chemical constituents during senescence (Collier and Thibodeau, 1995). In general, respiration may highest in incipiently senescent floret and floret respiration shows a climacteric like pattern falling during floret development then rising during floret senescence.

5.8 PETAL SENESCENCE:

The bio-chemical changes during petal senescence are presented in table-19, 20, 21, 22 and 23. The data recorded on protein, nucleic acid, total carbohydrate, starch, phenol, total anthocyanin and carotenoid content reveal that there was increase in water soluble protein but the total protein was decreased as compared to the protein content in fresh, fully grown petal. This decrease may be associated with hydrolysis of protein during senescence and decrease in synthesis or increase in degradation. Reduction in protein content is progressing through degradation to a mixture of smaller polypeptide and amino acid (Parups, 1971) and cause an increase in cell pH in the tissues.

The nucleic acid content was increased during petal senescence, since ethylene stimulates RNA synthesis in senescence that triggers for the synthesis of the hydrolytic enzyme.

The data presented in table-21 reveals that there was decrease in reducing sugar, increase in the non-reducing sugar but decrease in the total sugar during petal senescence as compared to the fresh, fully-grown petal. This decrease in reducing sugar because of translocation from the perianth to other organs at the senescence (Yamane, 1991). There was decrease in starch content, since in many higher plants, the principal end product of petal are carbohydrate such as starch and sucrose and during senescence hydrolysis of starch starts. Also, phenol content was increased might be due to the leakage of phenol from the vacuole and their consequent interaction with the remaining functional elements of the cells lead to senescence (Liebermann and Biale, 1956).

The total anthocyanin and carotenoid content and its constituents decreased during petal senescence due to increase in pH of the standard

vase solution. Since, high pH (4.40-4.50) result accumulation of NH_3 in the petal tissue when petal tissue begin to be utlilized as respiratory substrate shows less red as the petal tissue aged (Weinstein, 1957). This low pigmentation due to NH_3 formation limited by the carbohydrate content of the tissue (Kuc, 1964).

5.9 CORM PRODUCTION:

The perusal of data on corm characters is presented in table-16. It is apparent from data that increased in number of corms/plant, number of cormels/plant, mean weight of corm and cormels and diameter of corms were resulted by the effects pre-soaking kinetin, foliar GA_3 and pre-soaking + foliar spray kinetin and GA_3 at both the rates. Maximum number of corms and cormels per plant, mean weight of corms and cormels and diameter of the corm were found under T_{10} (pre-soaking + foliar spray GA_3 100ppm). The increase in corm production may be attributed mainly to higher number of leaves. Plant having more photosynthates which are ultimately translocated to the corm thereby, increase the corm size, corm weight, cormel number and cormel weight. These results are in close confirmity with the findings of Arora *et al.*, 1992; Leena *et al.*, 1992; Bhattacharjee, 1984 and Suh *et al.*, 1995, but these results contradict the findings of Preeti *et al.* (1997) elucidated that pre-planting treatment of bulbs of tuberose cv. Single with GA_3 (50, 100 or 200ppm) reduced the number of bulbs produced per plant irrespective of the treatments.

Experiment-2: Studies on the effect of pre-soaking and foliar spray of growth regulators on *Gladiolus grandiflorus* L. cv. Jessica.

5.10 PLANT GROWTH CHARACTERS:

The perusal of data (table-24) on number of days required for sprouting of corms and percentage of sprouting at 30 days after planting revealed that all the plant growth regulator treatments significantly reduced the number of days required for sprouting of corms and were observed minimum at T_{11} (pre-soaking + foliar spray GA_3 200ppm). Significant increase in the percentage of sprouting at 30 days after planting were also noticed and maximum percentage of sprouting was found under T_{11}. These results are in conformity with the findings of Choudhary, 1987; Davendra *et al.*, 1999 and Arora *et al.*, 1992.

5.11 PLANT HEIGHT AND LEAF AREA:

The result of the preceding chapter indicate that the effect of different treatments were found significant on various growth characters at all the stages of crop growth with respect to the plant and leaf area except number of leaves per plant during both the years of trial (table-25).

The maximum plant height were obtained with T_{10} (pre-saoking + foliar spray GA_3 100ppm) at all the stages of observation during 1999-2000 and 2000-2001. These findings are in line with the result of various workers viz. Leena *et al.*, 1992; Bhattacharjee, 1984; Dua *et al.*, 1984; Kumaran, 1993 and Mishra *et al.*, 1993.

Though the leaf number was not significant under various treatments. However, maximum leaf number was observed under T_4 (pre-soaking kinetin 25ppm) confirms the findings of Roychoudhary *et al.*, 1985. Whereas, increased leaf area was associated with T_{10} and T_{11}. These results are corroborate with the findings of Leena *et al.*, 1992 and Kumaran, 1993.

5.12 FLOWERING CHARACTERS:

The data recorded on flowering characters are presented in table-26 and 27. It is apparent from data that flowering behavior of gladiolus were also influenced markedly by GA_3 and kinetin treatments pre-soaking, foliar or pre-soaking + foliar spray. The minimum number of days required for spike initiation, maximum number of florets/spike and fresh weight of spike at the time of harvesting was obtained with T_{11} and T_{10}. Earliness in spike initiation may be due to the extension growth caused by increased photosynthesis with enhanced CO_2 fixation or GA_3 hastened the differentiation of floral primordia. Ginzburg (1974) confirmed GA_3 responsible for the assimilate movement towards the inflorescence at the expense of the gladiolus corm, which might have influenced early emergence of flower spike. These results are in agreement with the findings of Karguzel *et al.*, 1999; Reddy *et al.*, 1997; Dutta *et al.*, 1998; Mahesh and Mishra, 1993 and Das *et al.*, 1992.

5.13 PHYSIOLOGICAL CHANGES DURING VASE-LIFE:

The data recorded on physiological changes during vase-life are depicted in table-28, 29, 30 and 31. Daily elongation of cut spike, days to elongation of cut spike, length and diameter of floret, water uptake and loss and quality parameters of spike were influenced by lower concentration of GA_3 and kinetin when applied as pre-soaking, foliar and pre-soaking + foliar spray. All these characters were showed increased response which are associated with T_{10} (pre-soaking + foliar spray GA_3 100ppm) except maximum length of flower under T_{13} (pre-soaking + foliar spray kinetin 50ppm), number of days to senescence of basal floret under T_9 (foliar spray kinetin 50ppm). But overall enhanced response was observed with T_{10} (pre-soaking + foliar spray GA_3 100ppm) and T_{11} (pre-soaking + foliar spray GA_3 200ppm). These results are corroborate with the findings of Lukaszewska, 1995; Reddy *et al.*, 1997; Nagarjuna and Gowda, 1998; Dennis *et al.*, 1983; Mayak *et al.*, 1972; Lal *et al.*, 1990 and Nowak, 1989.

Increased elongation of spike associated with pre-soaking + foliar spray GA_3 at both the rates may be attributed due to the more water uptake but increased rate of elongation was observed over a certain period after that elongation was sustained might be due to high water potential maintained within the xylem vessels of cut spike or cells attain maximum enlargement.

Length and diameter of flower might have enhanced due to increase in the length of petals and pedicel, While decreased floret size associated with T_1 (control) may be due to a decreased rate of water uptake. This water deficit may be the result of increase in viscosity of vase solution and decreased transpiration rate due to the presence of sugar.

Increased water uptake and minimum water loss was observed under T_{10} and T_4. Since, sucrose in holding solution provides an energy source, also act as antidessicant and inclusion of 8-HQC prevent wilting due to an antitranspirant activity (Stoddard and Miller, 1962) and dessication of the flower stems by inhibiting vascular blockage and allowed greater water conductivity (Larson and Cromarty, 1967).

Quality parameters viz. increase in time required for opening of basal floret, number of days to senescence of basal floret, number of florets/spike open at the time of senescence of basal floret, number of florets/spike before stem collapse and longer vase-life were associated with T_{10} (pre-soaking + foliar spray GA_3 100ppm) except increase in number of days to senescence of basal floret was found under T9 (Foliar spray kinetin 50ppm). Enhancement in quality parameters may be due to the improved water balance in flowers throughout the vase-life by closing the stomata and reducing water loss (Marousky, 1971).

Longer vase-life was found under T_{10} (Pre-soaking + foliar spray GA_3 100ppm) when kept under standard vase solution (4 per cent sucrose and 200ppmm 8-HQC). The improvement in vase-life may be due to the translocation of sugar from vase solution via cut spike stem and accumulation in the florets, increases their osmotic concentration and improve their ability to absorb water and maintain turgidity (Halevy, 1976). The main effects of applied sugar on flower longevity results from their contribution to their osmotic adjustment of the flowers and also helps in

maintaining mitochondrial structure and function (Kaltaler and Steponkus, 1976) and delaying the onset of senescence (Mayak *et al.*, 1975) and maintaining the carbohydrate pool in petal (Rogers, 1973). The inclusion of 8-HQC in the vase solution extends the vase life, are considered to be the reduction of vascular blockage, increase in water uptake and lowering of water loss through transpiration (Marousky, 1968-69) and also helps in the acidification of vase solution due to presence of citrate ion which maintains the optimum pH. Thus, Waters (1966) indicated that no absolute method of measuring spike quality have been established, however gladiolus longevity and other quality can be measured as floret opening and senescence as well as fresh weight and the effect of GA_3 on translocation of assimilates, extended vase-life and delayed senescence may result from direct effect of GA_3 on cell senescence associated process may also affect membrane permeability and subsequent leakage of electrolytes and preserving membrane integrity.

5.14 BIO-CHEMICAL CHANGES DURING VASE-LIFE:

It is obvious from the findings obtained through various bio-chemical changes, signifies that to improve the longevity or enhancement of petal senescence various internal as well as external changes occurs, affected mainly due to the metabolic changes by various treatments while keeping in standard vase solution of 8-HQC + sucrose. The data observed by series of bio-chemical changes during the vase-life in table-32, 33, 34, 35, 36 and 37 showed decrease in enzyme activity, increase in water soluble protein, nucleic acid, reducing sugar, total sugars, decrease in starch and phenol and considerable increase in total anthocyanin and carotenoid were under T_{10} (pre-soaking + foliar spray GA_3 100ppm), whereas increased carotenoid content was associated with T_6 (foliar GA_3 100ppm).

Decrease in enzymatic activity is the result of the use of flower preservative, retarding peroxidation usually by neutralizing free radicals.

Increased protein content and nucleic acid may be ascribed through specific activities of the protein and RNA or maintenance of the DNA as a

functional template for DNA dependent synthesis of RNA. Also, synthesis of RNA is stimulated by the presence of GA_3.

The reducing sugar was also noticed higher under T_{10}, since it is predetermined fact that absorbed sucrose in petal are rapidly converted to reducing sugar which then accumulate in the corolla (Wagner, 1979). Whereas, decrease in starch content may be temporarily associated with rapid tissue expansion or increase in fresh weight of the spike and the quantitative disappearance of starch with a corresponding increase in glucose, fructose and to lesser extent sucrose and the starch hydrolysis provides osmotic solutes of water influx and cellular expansion.

During vase-life studies of cut gladiolus spike, the increased synthesis of anthocyanin and carotenoid was noticed and was found that enhanced pigmentation in petal due to higher concentration of peonidine with respect to delphinidine and pelargonidine and rapid accumulation of anthocyanin in petal has commonly been observed in the later stage of flower development (Davies *et al.*, 1993). The induction of anthocyanin synthesis requires the presence of sugar in the medium and sugars may serve as specific signals for the activation of specific genes, as cellular osmotic regulators, or as a general energy source of carbon metabolism in the developing flower (Delila *et al.*, 1997). Whereas, regarding carotenoid content, α-carotene content was maximum than β-carotene content indicates that reddish yellow pigmentation in gladiolus floret due to the presence of peonidine and α-carotene pigment constituent.

These results are in close conformity with the findings of Donald, 1997; Soleimani, 1968; Nichols, 1979; Bieleski, 1993; Kaltaler and Steponkus, 1974; Paulin, 1980, Yamane *et al.*, 1991; Weinstein, 1957; Celikel and Doorn, 1995; Justisen *et al.*, 1997; Zeislin and Halevy, 1969; Neilson and Bloor, 1997; Suneetha and Kumar, 1998; Ichimura, 1998 and Kazakidon and Burrage, 1994.

5.15 pH:

Data recorded on pH changes over the initial standard value of 3.5 is presented in table-38 reveals that at the termination of vase-life of cut gladiolus, the minimum pH was associated with T_{10} (pre-soaking + foliar spray GA_3 100ppm). Low pH of flower preservative solution results in improved keeping life. A strongly acid condition may inhibit endogenous enzyme essential for the stem plugging process, may also inhibit microbial growth. These results are in line with the findings of Reist, 1977; Pokorny and Kamp, 1953; Marousky, 1971 and Phavaphutanan and Ketsa, 1989. Whereas, this result contradict with the findings of Jones and Meagan (1993) revealed that pH of the 8-HQC solution did not appear to affect vase-life of rose, gerbera, gypsophila, carnation and chrysanthemum.

5.16 ETHYLENE AND RESPIRATION:

Since, gladiolus is considered that as ethylene insensitive flower (Reid and Wu, 1991) but ethylene plays a major role in senescence of cut spike during the vase-life. The data recorded on ethylene production and respiration are presented in table-40 and 41 reveal that low rate of ethylene production during phase-1, subsequently increased during phase-2 then after sharp decline in phase-3 were influenced by all the treatments. Since, time course of ethylene production follows a typical profile composed of three distinct phases, 1. Slow steady rate 2. An accelerated rises in maximum emanation and 3. A last phase in which production is declining. Thus, emanation might be influenced by changes either in the capacity of ethylene binding sites (Jerie *et al.*, 1978) or ethylene metabolism rate (Beyer, 1978). Minimum ethylene production was observed under T_{10}. An increase in the intercellular concentration in phase-2 leads to an increase in tonoplast permeability (Mayak *et al.*, 1977). Increased ethylene biosynthesis under T10 during the phase-2 may also be due to the lack of carbohydrate content and can be promoted due to the autocatalytic action. A change in hormonal balance during the senescence of flower, the level of cytokinin

reduced while ethylene and ABA increased (Halevy and Mayak, 1975) and also the biosynthesis of ethylene is promoted by the presence of free radicals or it may be that the ethylene burst, in turn produces free radicals, promotes the oxidative metabolism lead to senescence.

Like ethylene, the rate of respiration also followed the same trend and the magnitude of reduction in rate of respiration was highest under T_{10} as compared to the control. The rise in respiration rate at phase-2 may be suggested due to the formation of free radicals with high oxidation potential and this increase in respiration due to the climacteric stage of the flower and may be accompanied by an increase in fresh weight and cell size. Also, increased CO_2 concentration in the petal by increase in their rate of respiration and sucrose enhanced the rates of respiration. Rapid drop in respiration during phase-3 consistent with the poor export of sugars and amino acid. Since, respiration is required for degradation and metabolization of chemical constituents during senescence (Collier and Thibodeau, 1995). It has been established that the mitochondria of post climacteric (senescent) cells were still capable of oxidative phosphorylation and that the cells still perform anabolic functions, the synthesis of proteins, but in the final stage of senescence result from the leakage of phenols from the vacuole and their consequent interaction with the remaining functional element of the cells lead to senescence (Leibermann, 1956). In general, respiration may highest in incipiently senescent floret and floret respiration shows a climacteric like pattern falling during floret development then rising during floret senescence.

These results are in agreement with the findings of Apelbaum and Yang, 1981; Roh, 1990; Donald, 1997; Dennis *et al.*, 1983 and Marousky, 1969.

5.17 PETAL SENESCENCE:

The bio-chemical changes during petal senescence are presented in table-42, 43, 44, 45 and 46. The data recorded on protein, nucleic acid, total carbohydrate, starch, phenol, total anthocyanin and carotenoid content reveal that there was increase in water soluble protein but the total protein

was decreased as compared to the protein content in fresh, fully grown petal. This decrease may be associated with hydrolysis of protein during senescence and decrease in synthesis or increase in degradation. Reduction in protein content is progressing through degradation to a mixture of smaller polypeptide and amino acid (Parups, 1971) and cause an increase in cell pH in the tissues. These result confirms the findings of Celikel and Doorn, 1995; Donald, 1997; Jiang *et al.*, 1997 but this finding contradict the result of Su and Ye (1997) opined that soluble protein content increased initially and later decreased during vase-life of *Freesia refracta* cv. Auroia.

The nucleic acid content was increased during petal senescence, since ethylene stimulates RNA synthesis in senescence that triggers for the synthesis of the hydrolytic enzyme. This result contradict the findings of Matile and Winkerbach, 1971 and Fletcher and Osborne, 1965 but supports the findings of Soleimani (1968) opined that during senescence ratio of RNA/DNA decreased as the present investigation follows the same findings.

The data presented in table-44 reveals that there was decrease in reducing sugar, increase in the non-reducing sugar but decrease in the total sugar during petal senescence as compared to the fresh, fully-grown petal. This decrease in reducing sugar because of translocation from the perianth to other organs at the senescence (Yamane, 1991). These results are in close confirmity with the findings of Bhattacharjee, 1997a and Annonimous, 1996-1997 but contradict the findings of Kapchina (1989) revealed that at petal senescence quantity of reducing sugar increased. There was decrease in starch content, since in many higher plants, the principal end product of petal are carbohydrate such as starch and sucrose and during senescence hydrolysis of starch starts. Also, phenol content was increased might be due to the leakage of phenol from the vacuole and their consequent interaction with the remaining functional elements of the cells lead to senescence (Liebermann, 1956). These results corroborate with the findings of Ho and Nichols, 1977; Ramanuja Rao and Mohan Ram, 1979; Yamane *et al.*, 1991; Mayer and Friend, 1960 and Weinstein, 1957.

The total anthocyanin and carotenoid content and its constituents decreased during petal senescence due to increase in pH of the standard vase solution. Since, high pH (4.40-4.50) result accumulation of NH_3 in the

petal tissue when petal tissue begin to be utilized as respiratory substrate shows less red as the petal tissue aged (Weinstein, 1957). This low pigmentation due to NH_3 formation limited by the carbohydrate content of the tissue (Kuc, 1964). These results are in line with the findings of Justisen *et al.*, 1997; Merzlyak *et al.*, 1999 and Ichimura, 1998.

5.18 CORM PRODUCTION:

The perusal of data on corm characters is presented in table-39. It is apparent from data that increased in number of corms/plant, number of cormels/plant, mean weight of corm and cormels and diameter of corms were resulted by the effects pre-soaking kinetin, foliar GA_3 and pre-soaking + foliar spray kinetin and GA_3 at both the rates. Maximum number of corms and cormels per plant, mean weight of corms and cormels and diameter of the corm were found under T_{10} (pre-soaking + foliar spray GA_3 100ppm). The increase in corm production may be attributed mainly to higher number of leaves. Plant having more photosynthates which are ultimately translocated to the corm thereby, increase the corm size, corm weight, cormel number and cormel weight. These results are in close confirmity with the findings of Arora *et al.*, 1992; Leena *et al.*, 1992; Bhattacharjee, 1984 and Suh *et al.*, 1995, but these results contradict the findings of Preeti *et al.* (1997) elucidated that pre-planting treatment of bulbs of tuberose cv. Single with GA_3 (50, 100 or 200ppm) reduced the number of bulbs produced per plant irrespective of the treatments.

SUMMARY AND CONCLUSION...

The present investigation entitled' **Studies on the effect of VAM and growth regulators on *Gladiolus grandiflorus* L. cv. Jessica**' was carried out at Horticultural Research Farm, CCS Haryana Agricultural University, Hisar during year 1999-2000 and 2000-2001.

The experiment was laid out in Randomized Block Design for field investigation and Completely Randomized Design for laboratory experiment keeping thirteen treatment with or without V.A.M along with two plant growth regulators alone or in combination. The experiment was replicated thrice for field experiment and four for laboratory experiment during both the years. Observations on vegetative growth, flowering, physiological and biochemical changes during vase-life and corm production characters were recorded. Comparison of the plant growth regulator treatments with or without V.A.M using parameters viz. plant height, spike length, corm yield etc., also provided reliable measures of effectiveness of plant growth regulators treatments. Salient findings of the experiment are summarized.

Experiment 1. Studies on the effect of VAM and growth regulators on *Gladiolus grandiflorus* L. cv. Jessica.

1. Vegetative parameters viz. Number of days required for sprouting of corms, Percentage of sprouting 30 days after planting, plant height, number of leaves per plant and leaf area were influenced by various plant growth regulators alone or in combination with V.A.M. All the plant growth regulators in association with V.A.M treatments were significantly superior to control (T_1) in enhancing these characters. The treatments T_{12} (presoaking + foliar kinetin 50 ppm) followed by T_{10} (presoaking + foliar GA_3 100 ppm) and T_4 (presoaking kinetin 50 ppm) were found more effective in enhancing the plant growth. Higher concentration of either of the two plant growth regulators in association with V.A.M did not vary significantly over the lower concentration.

2. The minimum number of days required for spike initiation, maximum number.of florets per spike and maximum fresh weight of spike at the time of harvesting were associated with T_{12} (presoaking + foliar kinetin 50 ppm). Also; maximum fresh weight changes in cut spikes and number of days upto which fresh weight changes in spikes continued was significantly enhanced with T_{12} (presoaking + foliar kinetin 50 ppm).

3. Maximum elongation of flower spike and duration of elongation, maximum length and diameter of flower, increased water uptake and decreased water loss were recorded with T_{12} (presoaking + foliar kinetin 50 ppm) followed by T_{13} (presoaking + foliar kinetin 100 ppm) and T_{10} (presoaking + foliar $GA_3$100 ppm). In some parameters higher concentration showed remarkable response with non-significant differences with lower concentration and kinetin application with V.A.M was more effective than GA_3 regardless of the method of applications.

4. Quality parameters viz. Maximum days for opening of basal floret, days to senescence of basal floret, more number of opened florets per spike, maximum number of opened florets per spike before wilting or stem collapse and better longevity were related with V.A.M in combination with kinetin (presoaking + foliar kinetin 50 ppm) followed by T_{10} (presoaking + foliar GA_3 100 ppm in association with V.A.M). Kinetin + V.A.M particularly under combined application and presoaking alone proved better than GA_3 + V.A.M.
Regardless longevity, combined application of kinetin + V.A.M was followed by combined application of GA_3 + V.A.M, foliar (GA_3 + V.A.M), presoaking of Kinetin + V.A.M and presoaking of GA + V.A.M, specifically at lower concentration proved most effective.

5. Activity of peroxidase and polyphenol oxidase was significantly decreased under various treatments over the control. The magnitude of the activity was higher in case of polyphenol oxidase and minimum

enzyme activity was noticed under T_{12} (presoaking + foliar kinetin 50 ppm). Foliar application of kinetin + V.A.M also affect the activity of either of the two enzymes when compared with control.

6. Significant increase in water soluble protein, in contrast magnitude of reduction in water insoluble proteins under T_{12} (presoaking + foliar kinetin 50 ppm) and total proteins under T_7 (foliar GA_3 200 ppm) was observed. However, T_{12} (presoaking + foliar kinetin 50 ppm) was the most effective in enhancing the protein content. Increase in concentration of the plant growth regulators over the lower rate did not yield additional response.

7. Presoaking + foliar application of kinetin in association with V.A.M proved increase in RNA and DNA content in fresh petals and showed most effective and was significantly higher than all other treatments.

8. Increase in reducing sugars, total sugars and starch and decline in non reducing sugars and phenol content in fresh petals were most pronounced under T_{12} (presoaking + foliar kinetin 50 ppm) followed by T_{10} (presoaking + foliar GA_3 100 ppm) and T_6 (foliar GA_3 100 ppm). Incase of phenol content could not give further response over their concentration.

9. Pigments like anthocyanin and carotenoid content, T_{12} (presoaking + foliar kinetin 50 ppm) was the most effective in enhancing all the pigment constituents followed by T_{13} (presoaking + foliar kinetin 100 ppm) and T_{10} (presoaking + foliar GA_3 100 ppm). Higher concentration of pelargonidine and α-carotene content indicates the reddish yellow pigmentation in gladiolus floret.

10. Presoaking + foliar application of plant growth regulators in combination with V.A.M particularly under kinetin gave the most pronounced response with respect to reduction in pH. Low pH

throughout the vase-life under T_{12} (presoaking + foliar kinetin 50 ppm) registered maximum longevity, indicates that 8-HQC maintain the pH and inhibits the resulting increase in pH against the initial standard pH value of 3.5.

11. The number of corm and cormels, diameter of corms, weight of corm and cormels, were significantly influenced by different plant growth regulators and all the treatment resulted in a significant increase in the corm attributes as compared to the control. The maximum number of corms per plant, mean weight of cormels and diameter of the corm were observed under T_{10} (presoaking + foliar GA_3 100 ppm) followed by T_4 (presoaking kinetin 50 ppm) and T_6 (presoaking GA_3 100 ppm). Presoaking with GA_3 + V.A.M at both the rates did not prove effective except for the mean weight of the corm. Most of the treatment involving presoaking + foliar application of plant growth regulators + V.A.M did not produce variable response in comparison to the each other.

12. Combined application of kinetin i.e. T_{12} was the most effective in checking ethylene evolution and magnitude of the reduction in rate of respiration was highest under T_{12}. Higher concentration of either of two plant growth regulators in combination with V.A.M did not produce additional response irrespective of the phases of observations.

13. At the time of petal senescence of florets, water-soluble proteins increased but the total protein content was decreased as compared to the protein content in fresh, fully-grown petal.

Nucleic acid content and total sugars and phenol content increased while, there was decline in starch content and low pigmentation was noticed at the time of petal senescence as compared to all the attributes of fresh, fully-grown petal.

Experiment 2. Studies on the effect of presoaking and foliar spray of growth regulators on *Gladiolus grandiflorus* L. cv. Jessica.

1. Significant reduction in number of days required for sprouting of corms and increase in percentage of sprouting at 30 days after planting were found under T_{11} (presoaking + foliar GA_3 200ppm) whereas, maximum plant height was concerned with T_{10} (presoaking + foliar GA_3 100ppm) and increased leaf area was associated with T_{10} (presoaking + foliar GA_3 100ppm) followed by T_{11} (presoaking + foliar GA_3 200ppm). This infers that lower concentration was effective with vegetative parameters.

2. Flowering characters viz. Minimum number of days required for spike initiation, maximum number of florets per spike and fresh weight at the time of harvesting were obtained with T_{11} (presoaking + foliar GA_3 200ppm) and T_{10} (presoaking + foliar GA_3 100ppm).

3. All the physiological changes during vase-life of cut gladiolus spike while keeping in standard vase solution (4per cent + 200 ppm 8-HQC) were influenced by lower concentration of GA_3 i.eT_{10} (presoaking + foliar GA_3 100ppm).

4. Decrease in enzymatic activity and starch content, increase in protein content, nucleic acid and reducing sugar. Also, enhanced pigmentation of fresh fully grown petals indicate that reddish yellow pigmentation in gladiolus floret due to the presence of peonidine and α-carotene pigment constituents and augmented by all the biochemical attributes, observed under T_{10} (presoaking + foliar GA_3 100ppm).

5. At the termination of vase-life of cut gladiolus, the minimum pH was observed under T_{10} (presoaking + foliar GA_3 100ppm) signifies that cut gladiolus treated with presoaking + foliar GA_3 100ppm, ehile

keeping in standard vase solution (4 per cent sucrose + 200 ppm 8-HQC) maintains the pH throughout the vase-life and responsible for linger longevity of cut gladiolus.

6. The number of corm and cormels, diameter of corms, weight of corm and cormels, were significantly influenced by different plant growth regulators and all the treatment resulted in a significant increase in the corm attributes as compared to the control. The maximum number of corms per plant, mean weight of cormels and diameter of the corm were observed under T_{10} (presoaking + foliar GA_3 100 ppm).

7. Combined application of kinetin i.e. T_{10} was the most effective in checking ethylene evolution and magnitude of the reduction in rate of respiration was highest under T_{10}. Higher concentration of either of two plant growth regulators in combination with V.A.M did not produce additional response irrespective of the phases of observations.

8. At the time of petal senescence of florets, water-soluble proteins increased but the total protein content was decreased as compared to the protein content in fresh, fully-grown petal.
Nucleic acid content and total sugars and phenol content increased but the ratio of RNA/DNA was decreased. While, there was decline in starch content and low pigmentation was noticed at the time of petal senescence as compared to all the attributes of fresh, fully-grown petal.

Taking in to account of all these aspects of VAM and plant growth regulator effects, it is concluded that combined application of treatments were found to be more effective in enhancing plant growth, increasing the longevity by affecting various physiological and bio-chemical changes and corm yield than their separate application and on the basis of this fact, the treatment T_{12} (Pre-soaking + Foliar kinetin 50ppm in association with VAM) and T_{10} (Pre-soaking + Foliar GA_3

100ppm) were found most effective for enhancement of all the attributes responsible for the better longevity.

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Abstract

Title of thesis	: Studies on the effect of VAM and Growth regulators on *Gladiolus grandiflorus* L. cv. Jessica.
Full Name of Degree Holder	: SUNIL KUMAR
Admission Number	: 97A45D
Title of Degree	: Ph.D (Horticulture)
Name and Address of Major Advisor	: Dr. A. K. Gupta, Department of Horticulture, CCS Haryana Agricultural University, Hisar-125 004.
Degree Awarding University/Institute	: CCS Haryana Agricultural University
Year of Award of Degree	: 2002
Major Subject	: Horticulture
Total Number of pages in thesis	: 164 + xxi
Number of words in the Abstract	: Approx. 300

A field as well as lab experiment was conducted to assess the effect of different plant growth regulator treatments with or without VAM on growth, flowering and corm yield of gladiolus. These parameters provided reliable measures of effectiveness of plant growth regulator treatments. The changes occur in the cut flowers, when placed in standard solution of 8-HQC (200 ppm) + sucrose (4 per cent) during vase-life and the factors are more responsible for its better longevity and cause for senescence, were also investigated during the experimental period. Results showed that during both the years of observations, plant growth, flowering, physiological and bio-chemical changes during vase-life and corm yield of gladiolus were significantly increased by different plant growth regulator treatments as compared to control. Pre-soaking + foliar kinetin 50ppm in association with VAM proved significant response with respect to these parameters except corm yield which was influenced by pre-soaking + foliar GA_3 100ppm in combination with VAM. On the other hand, among the treatments in absence of VAM, pre-soaking +

foliar GA_3 100ppm were found to be the best during both the years and enhanced vase-life considerably.

The bio-chemical changes in the fresh, fully grown petals and during petal senescence were also studied. Decrease in total protein may be due to decrease in synthesis or increase in degradation, starch content and anthocyanin and carotenoid contents due to increase in pH of the standard vase solution. While, increase in water-soluble protein, nucleic acid (RNA and DNA content) might be due to the ethylene evolution stimulates RNA synthesis which triggers for the synthesis of the hydrolytic enzyme, total sugar content and phenol content during petal senescence as compared to fresh, fully grown petals were noticed and highlights the probable cause of petal senescence resulting poor longevity in the vases.

Low rate of ethylene evolution and respiration rate during phase-1 subsequently increase during phase-2 might be due to the formation of free radicals with high oxidation potential, then after sharp decline in phase-3, signifies cause of the petal senescence.

During the whole experimentation, it was observed that lower concentration of plant growth regulators with or without VAM incorporation within the soil vicinity proved better response than their higher doses.

Major Advisor

Signature of Student

Head of Department