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Research Article

EFFECT OF MACRONUTRIENTS AND PLANT GROWTH HORMONES FOR THE *IN VITRO* FORMATION OF CORMLETS OF *GLADIOLUS PACIFICA*

Nasen Kundang, Varun Kumar and *Sandeep Kumar
Department of Biotechnology, NIET, NIMS University, Jaipur-303121, Rajasthan, India
**Author for Correspondence*

ABSTRACT
Gladiolus known as queen amongst the bulbous flower with magnificent inflorescence belongs to the family of Iridaceae which is mostly propagated by "corm". Propagation via seed is very slow. Experiments were conducted to evaluate the potential of *in-vitro* propagation techniques and to check the effects of nitrogen, hormone and calcium on formation of *Gladiolus pacifica* cormlets. Vertical slice of corm was taken as explant for initiation of cultures of *Gladiolus pacifica*, which were cultured on MS media with different vitamins and growth hormones. Maximum shoots initiation was found on MS medium with NAA (0.5 mg/l) and BAP (2.0 mg/l). After 8 weeks, cultures showed healthy proliferation of adventitious shoots. The *in vitro* grown shoots are transferred to liquid media with various concentrations of hormones, calcium and nitrogen, individually and in combination with/without activated charcoal to check its effect on cormlet formation using coir as matrix. Average eight numbers of cormlets on effects of nitrogen with activated charcoal were produced on MS medium supplemented with the combination of NH_4NO_3 + KNO_3 (14N/l + 100N/1, 28N/1 + 200N/1, 42N/1 + 300N/1). The best combination on effect of hormone was found on IAA + α-NAA (1.0 mg/l +1.0 mg/l, 0.5 mg/l + 0.5 mg/l). Maximum number of cormlet on effect of calcium was obtained at 5 g/l conc. of calcium chloride.
***Abbreviations*:** IAA: Indole acetic acid, αNAA: 1-Napthalene Acetic Acid, BAP: 6-Benzyl Amino Purine, MS Media: Murashige & Skoog media, SDDW: Sterilized Double Distilled Water, AC: Activated Charcoal

***Keywords**: Gladiolus pacifica, Corm, In Vitro Culture*

INTRODUCTION
Gladiolus known as queen amongst the bulbous flower with magnificent inflorescence belongs to the family of 'Iridaceae'. It is propagated by "corm" and one mother corm generally produces one daughter corm of standard size and few cormlets.
Gladiolus is rated as the most popular flower in the world from the commercial viewpoint (Cohat, 1993). These are attractive, perennial cormous flowering plants and are semihardy in temperate climates (Nanus is hardy to Zones 5-8).
The *Gladiolus* is best known for interior decorative work. Their stems are generally unbranched, producing 1 to 9 narrow, sword-shaped, longitudinal grooved leaves. *Gladiolus* is propagated by seeds as well as vegetative means i.e., by corms. Some of the largest grandiflorus cultivars can get up to 5.5 ft. tall, while some miniatures don't reach 90cm height.
Some countries production of gladiolus supports the nation economy as Coetzee (2000) stated that the Netherlands earns more from flower than South Africa earns from gold. The genus *Gladiolus* contains about 300 species, the World Checklist of Selected Plant Families had over 276 species in 1988. As of February 2017, it accepted 300 species. There are 260 species of *Gladiolus* endemic in southern Africa, and 76 in tropical Africa.
About 10 species are native to Eurasia. Based on the geographical origin, *gladiolus* species are categorised into four group viz., Eurasian group, East African group, Natalensis group and South African Cape species.
They have very slow rate propagation via seeds. They are also used as food plants by the larvae of some *Lepidoptera* species and are borne to infection by soil-borne fungus, *Fusarium oxysporum f.* sp. Micro

propagation and other *in vitro* techniques have been used for plants which present particular problems in conventional horticulture (Fay, 1992). Which is why the potential use of *in vitro* micro propagation for mass production of cormlets comes to play. *In vitro* production of *gladiolus* cormlets is relatively time minimizing with greater number of cormlets and genetic modification can be achieve.

MATERIALS AND METHODS

Preparation of Explant and Sterilization: *In vivo* produced corms of *Gladiolus pacifica* were used as explant. *Gladiolus pacifica* 'white' corms were washed thoroughly under running tap water for 30 min, soaked in liquid detergent (Tween 20) for 5 min, rinsed 5-6 times with sterilized double distilled water (SDDW) and then with ethanol (70%) for 40s. After two rinses with sterilized double distilled water, the corms was disinfected with a 0.1% aqueous solution of $HgCl_2$ for 10 min. and rinsed 6 times in SDDW. All the sterilization steps except washing with tap water, was done in laminar cabinet.

***Media Formulation*:** After sterilization, various slices of *Gladiolus* corm were taken as explant for initiation of culture. The corm slices were cultured and maintained on Murashige and Skoog media (1962) supplemented with various concentration of plant growth regulators like NAA(0-1mg/l) and BAP(0-10mg/l). Cultures were maintained at 24±2ºC with 24 hr light period. Sub-cultured after 6-8 weeks in the same media concentrations that is used for initiation of culture, for the multiplication of culture. Maximum adventitious shoots initiation was obtained on MS medium with α-NAA (0.5 mg/l) and BAP (2.0 mg/l).

To Check the Effect of Nitrogen on Formation of Cormlets (with/without Activated Charcoal): *In vitro* grown *Gladiolus pacifica* shoots were subsequently placed in culture tubes containing MS + BAP (2.0 mg/l) + NAA (0.5 mg/l) + sucrose (3%) + KNO_3 (100-400 N/l) + NH_4NO_3 (14-56 N/l) with sterile coir, as matrix, 3 sets with activated charcoal (2%) and 3 sets without activated charcoal. The data were recorded on weekly intervals.

To Check the Effect of Hormone on Formation of Cormlets with/without Activated Charcoal: Two auxins (NAA and IAA) were tested to see their effect on *in vitro* cormlet formation. MS medium was supplemented with different concentrations of IAA (0 to 1.0 mg/l) and NAA (0 to 2.0 mg/l) alone as well as in combinations with 3 sets each.

To Check the Effect of Calcium Sources: The basal MS medium was supplemented with calcium chloride without activated charcoal. Data recorded on MS + NAA (0.5mg/l) + BAP (2.0mg/l) + $CaCl_2$(0-10.0g/l)

Data Analysis

Data analysis was done by taking the mean and standard deviation of the different sets of cultures of different parameters studied.

RESULTS AND DISCUSSION

Vertical slices of corm were taken as explant for initiation of cultures of *Gladiolus pacifica*. After sterilization the corm slices were cultured on basal medium supplemented with various compositions of NAA and BAP. Maximum adventitious shoots initiation was obtained on MS medium with NAA (0.5 mg/l) and BAP (2.0 mg/l). After 6-8 weeks, cultures showed healthy proliferation of shoots. For further experimentation these shoots were subcultured on the same medium. In present study, the *in vitro* maintained shoots of *Gladiolus pacifica* were used and the effect of different media ingredients (such as nitrogen, calcium and growth hormones) were studied on the process of *in vitro* formation of cormlet.

Kamo (1994) reported that callus initiated from explant in cytokinin redifferentiated shoots readily. The presence of activated charcoal had a beneficial influence on both shoot regeneration and multiplication of shoots. For efficient micro propagation of *G. pacifica* only the medium containing 2mg/l BAP sustained a good growth of the shoots along with their multiplication and few cormlets formation, in a similar way to the results described for two *Gladiolus* varieties (Rao *et al.,* 1991a). Corm development was stimulated by increased concentrations of sucrose as it was demonstrated for some cultivars of *G. hybridus* (Goo and Kim, 1994).

Ahmad *et al.,* (2000), also studied the effect of basal medium with the varying concentrations of BAP, IBA and NAA on initiation and establishment of cultures of *Gladiolus*, a increase concentration of BAP against a decreasing concentration of NAA stimulated the shoot and cormel proliferation.

***Effect of Nitrogen on Formation of Cormlets with Activated Charcoal*:** The effect of ammonium nitrate and potassium nitrate was tested on the process of cormlet formation. Ammonium nitrate (14N/1 to 56N/1) and potassium nitrate (100 N/1 to 400N/1) were added in alone and in combination both along with activated charcoal (2%). After the first week upto sixth week of culture, the length and the number of shoots was found increased. No effect was seen on formation of cormlets during the first week while the second week and the fourth week observations showed the formation of cormlets. Significant increase in the formation of roots as well as in the cormlets was observed in the six weeks cultures. The maximum number of cormlet were produced on MS medium supplemented with the combination of NH_4NO_3 + KNO_3 (14N/l + 100N/1, 28N/1 + 200N/1, 42N/1 + 300N/1). Average eight numbers of cormlets were produced on these combinations. The experiment resulted out the best type. It is evident from this experiment that the nitrogen sources play a good role in the formation of cormlet.

Effect of Nitrogen on Formation of Cormlets without Activated Charcoal:

The permutaion of nitrogen on the formation of cormlets on MS medim along with BAP (2.0 mg/l) and NAA (0.5mg/l) was recorded. Results were significantly different from the previous experiment. Maximum number of cormlets was obtained at four different combinations of NH_4NO_3 + KNO_3 (14N/1 + 100N/1, 28N/1 + 200N/1, 42N/1 + 300N/1, 56N/1 + 400N/1) of which 42N/1 + 300N/1 ruled out best by forming 20 cormlets on eight week culture.

Effect of nitrogen on micropropagation of *Stevia rebaudiana (BERTONI)* was showed by Kumar *et al.*, (2013). The use of activated charcoal and auxins to improve rooting was also supported by the results of Sairkar *et al.,* (2009) as there was significant increase in percentage of medium for rooting was also supported by results of Arya *et al.,* (2012). Their results of study showed that no rooting occurred without plant growth regulators and presence of auxins increased the percentage of rooting in multiplied shoots. That way, we were able to develop an efficient *in vitro* technology for the micro propagation and multiplication of *G. pacifica*. Such a technology might be used, after molecular analysis of genetic stability of *in vitro* clones, for the restoration of natural populations into the habitats where this species became extinct.

Effect of Hormone on Formation on Cormlets without Activated Charcoal: Plant growth regulators certainly affect the *in vitro* response in various ways. Rani *et al.,* (2016), showed the effect of growth regulators on *in vitro* organogenesis of three cultivars of tomato (*Lycopersicon esculentum mill*.). In the present study the two auxins (α-NAA and IAA) were tested to see their effect on *in vitro* cormlet formation. MS medium (basal) was supplemented with different concentrations of IAA (0 to 1.0mg/l) and α-NAA (0 to 2.0 mg/l) alone as well as in combinations. No effect on cormlets formation was seen up to fourth week of culture duration. In sixth and the eight week of culture period, few concentrations of media showed the cormlets formation. The best combination was found to be IAA + α-NAA (0.5 mg/l +1.0 mg/l, 0.5 mg/l + 0.5 mg/l) resulting in 9 and 15 no. of cormlets formation.

***Effect of Hormone on Formation on Cormlets with Activated Charcoal*:** Effect of activated charcoal (2%) along with PGR (IAA and α-NAA alone and in combinations) was studied. Significant effect was seen in the texture of cultures. With increases in the concentrations of IAA and α-NAA the root formation was enhanced but no effect on cormlets formation was observed.

However, number of shoots greatly increased with the addition of cytokinins. Aftab *et al.,* (2008) reported more number of shoots (20) per culture vessel from cormlets on MS supplemented with BAP (2mg/l). However, this report did not mention the variety and size of cormlets used for shoot regeneration; hence, it is difficult to justify the results. Priyakumari and Sheela (2005) recorded the highest number of shoots (4) from axillary buds on MS medium supplemented with BAP+NAA (0.4+0.5 mg/l) in *Gladiolus pacifica*.

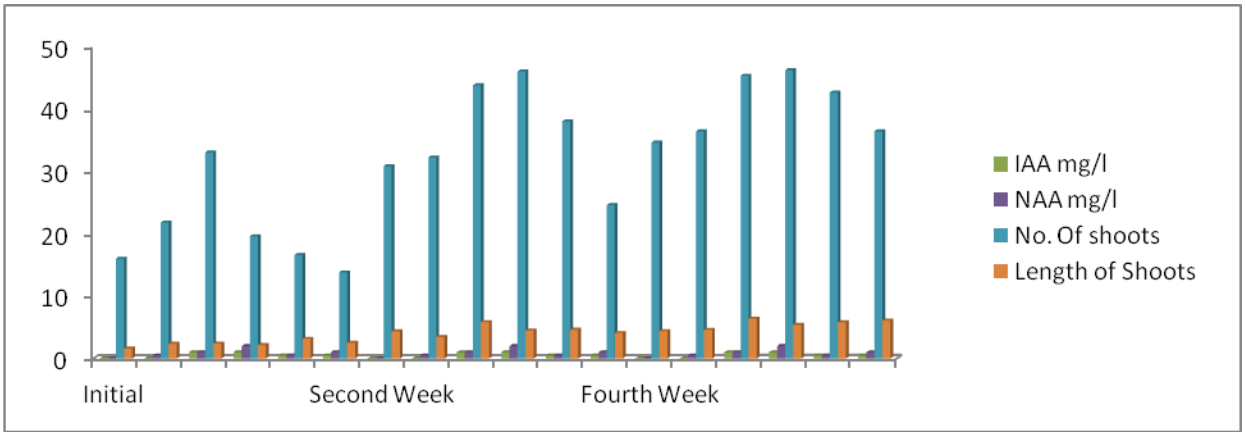
Effect of Calcium Chloride on Formation of Cormlets: Calcium is associated to the process such as membrane structure and function, ion uptake, reactions with growth regulators and enzymatic activation

via calmodulin (Malavolta *et al.*, 1997). The structural function of calcium is characterized by the use in the synthesis of new cell wall, particularly in the middle lamellae that separate newly divided cells (Taiz and Zeiger, 2006). To see the effect of calcium sources the basal MS medium was supplemented with calcium chloride devoid of activated charcoal. Results were significantly different from the previous experiment. By the fourth week, cormlet no. increased significantly in all calcium chloride concentrations. Maximum number of cormlets (22 cormlets) at 5 g/l conc. of calcium chloride was obtained at six week old culture with MS media + NAA(0.5 mg/l) + BAP(2.0 mg/l) and rooted shoots produced cormlets within six weeks.

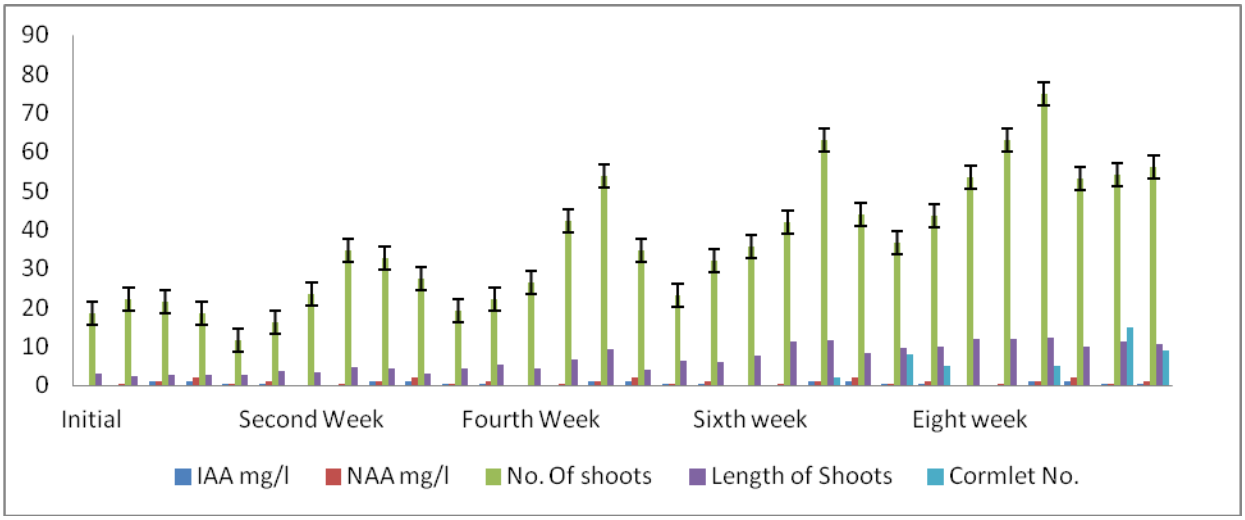
Figure 1: Multiplication of Shoots of *Gladiolus on MS with BAP and α-NAA*

Figure 2: Multiple Shoots on MS Medium with BAP and α-NAA after 8 Weeks

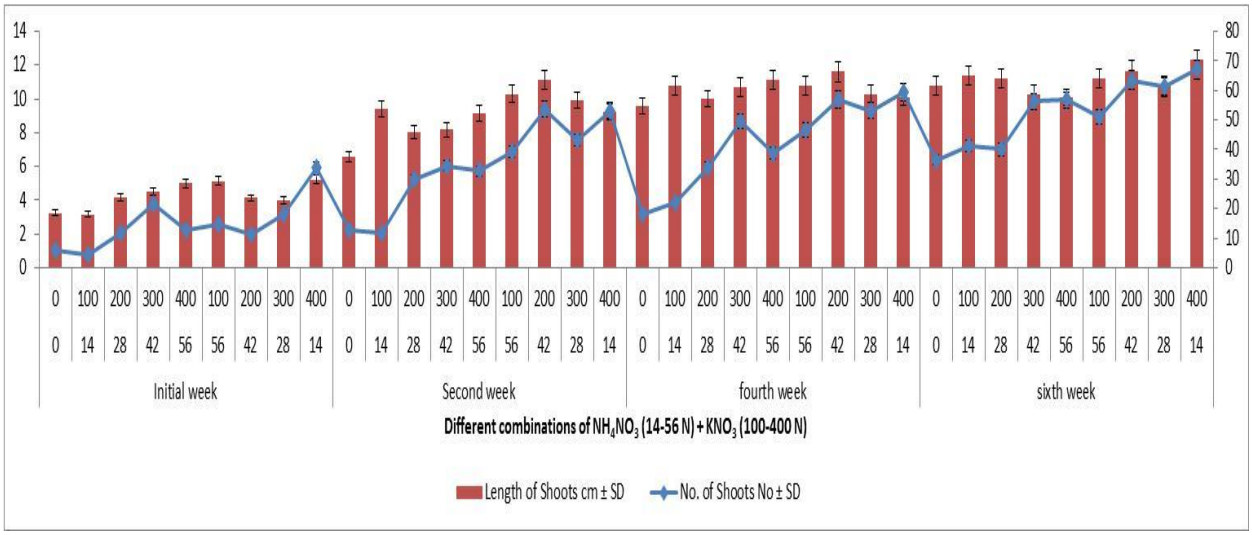
Figure 3: Formation of Cormlets on MS Liquid Media with BAP, α-NAA and Coconut Coir (Matrix)



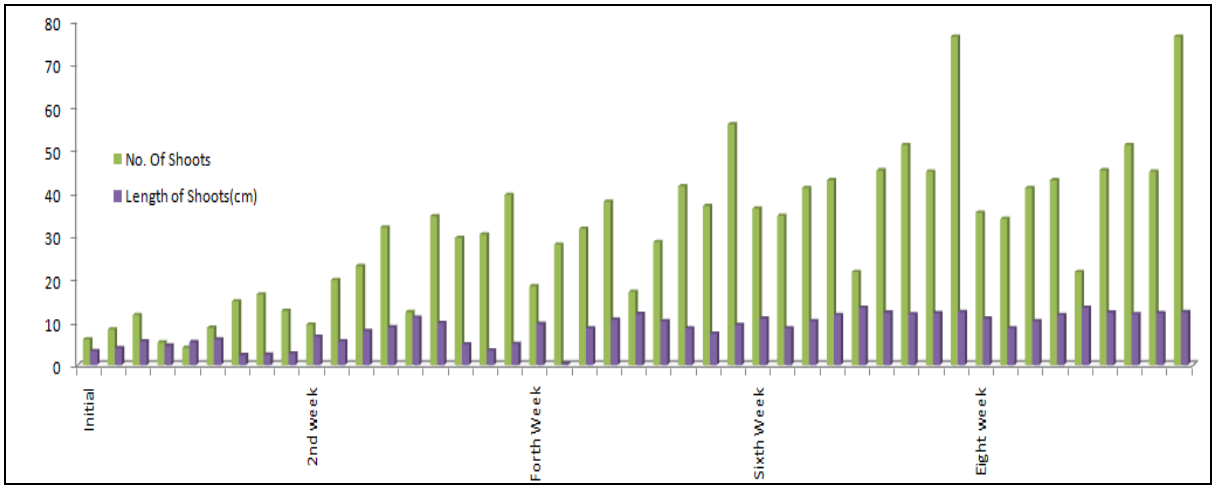
Graph 1: Effect of Hormone on Formation of Cormlets with Activated Charcoal



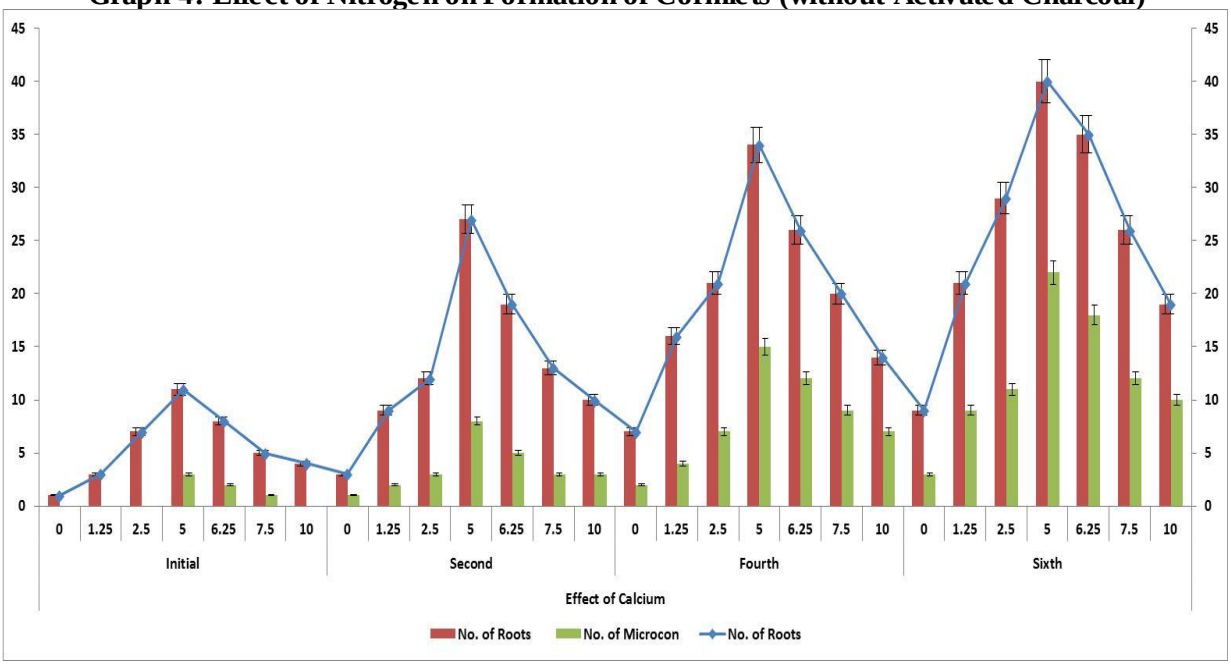
Graph 2: Effect of Hormone on Formation of Cormlets without Activated Charcoal



Graph 3: Effect of Nitrogen on Formation of Cormlet (with Activated Charcoal)



Graph 4: Effect of Nitrogen on Formation of Cormlets (without Activated Charcoal)



Graph 5: Effect of Calcium Concentration on Formation of Cormlet

Conclusion

It is concluded that corm and cormlet production can be achieve through simple MS media supplemented with various plant growth hormones. Hormones IAA(0.5mg/l) + NAA(0.5mg/l) combination resulted 15 no. of cormlets formation in eight week culture without activated charcoal but no corm formation with activated charcoal, they show more root formation. Use of activated charcoal enhance the formation of *Gladiolus pacifica* cormlets as compared with media deficient in activated charcoal. It is also evident from this experiment that the nitrogen and calcium sources play an important role in the formation of cormlets. Maximum number of cormlets (20 cormlets) was obtained at 42N/1 + 300N/1 of NH_4NO_3 + KNO_3. It was also concluded that the increasing calcium chloride level (5g/l) in the culture medium increases tissue calcium ion content and increase the cormlets numbers (22 cormlets) and size of cormlets.

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