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Using of Uv Light Type C and Growth Regulators in Propagation and Cormels Formation in *Gladiolus* Spp

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Abstract

Micropropagation of *Gladiolus spp*. (Primulinus) from axillary buds of flowers stalk as a source of explants. Axillary buds were sterilized under aseptic conditions and cultured in (MS) media supplemented with various levels of growth regulators (BA, IBA). The plantings were taken and exposed to UV light type C radiation with length 254 nanometer at different periods of time (30, 60, 90 second) . The effect of BA on the vegetative multiplication of buds was studied with concentrations (0.0, 0.25, 0.50 and 0.75) mg / liter, the best concentration for multibecation was 0.5 mg l. IBA with concentrations (0.0, 0.25, 0.50 and 1) mg / liter were used for roting and cormels formation, the best result present at the concentrations 0.50 mg/L. The result showed that UV light at time of exposure 60 sec gave the highest response to multibecation and cormels formation.

Keywords: UV light type C, growth regulators, *Gladiolus* spp

Introduction

Gladiolus is a bulbous herbaceous plant from tropical to subtropical climate (Barbosa 2011). Gladiolus is an excellent cut flower grown all over the world for its spikes with florets of massive form, brilliant colors, attractive shapes, varying sizes and long shelf life. However, major constraint for gladiolus cultivation is the corm dormancy. Plant growth regulators (PGRs) play an important role in breaking dormancy and promote more number of quality corm and cormel productions in gladiolus (Rashid, 2018). *In-vitro* techniques are applicable for the propagation of corm producing species. These techniques are adopted at commercial level in order to fulfill supply

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gap of huge demand. A number of *in-vitro* protocols have been developed for regeneration of Gladiolus plantlets using different media by using various explants sources of the plant (Memon *et al*., 2016). Different sours of explants were used to propagation of gladules cormls with different the chance such as tissue clutter with different of growth regulators and mutagenesis using uv light Sinha and Roy, various explants such as nodal buds showed a successful in- vitro propagation of Gladiolus cormels . (2002) Memon et al., (2010).

The aim of this research is to improve the propagation of Gladiolus bulbs and increasing the number of cormels using tissue culture technic and UV light as a mutagenesis sours.

Materials and methods

Gladiolus Primulinus where the flowers that purchased from markets. The work was carried out in the plant tissue culture lab, Institute of Genetic Engineering and Biotechnology and plant tissue culture lap, affiliated to the municipality of Baghdad during the period 15/8/2020 - 14\5\2021.

Surface sterilization

The explants were washed with running water for 15-20 minutes to get rid of dust and materials attached to them, and the axillary buds were separated from them inside the Laminar air flow-cabinet, the explants were immersed with 70% ethyl alcohol C2H5OH for one minute with continues stirring, then washed with sterile distilled water for three time and for 5 minutes to ensure removal of the harmful effect of alcohol, after that it was sterilized with sodium hypochlorate solution. NaOCl (Clorox minor solution) 2% for 10 minutes, the explants were washed with sterile distilled water three times to remove the effect of the Clorox minor, All the steps of sterilization were carried out under aseptic conditions using laminar air

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flow cabinet. Explants were transferred into sterilized Petri dishes having sterile filter papers to remove excess water (Ramgareeb *et al*., 2001).

Preparation of plant tissue culture medium

Murashige and Skoog (MS) medium was used with 4.43 g /l (Murashige and Skoog, 1962) components were prepared , supplemented vitamins , 30 g/l sucrose, 100 g /l Myoinositol And growth regulators (auxins and cytokinins) at the required concentrations according to the type of experiment.

The pH of the medium was adjusted to 5.8 using 1N NaOH or 1N HCl, then 7 g/L agar was added to the medium. For the good melting and mixing the mixture, the nutrient medium was placed on the magnetic stirrer / Hotplate device at a temperature of 95-90 ° C. Distribute the medium in glass containers with dimensions of 5 cm in diameter and 10 cm in height. The culture medium sterilized in autoclaved at a pressure of 1.04 kg/cm, 121.C, for 20 min., and then left at room temperature until use (Murashige and Skoog, 1962).

Initiation stage:

After sterilization was completed, the buds were transferred individually to a petri dish and then their ends that were damaged by the use of scalpel were cut so that the axillary buds became 1-2 cm and 0.5-1 cm, diameter and then the buds were transferred to culture media supplemented with BA with different concertations (0 , 1 , 1.5 , 2) and were incubated at 24-26 Cº under fluorescent light with 1000 lux intensity for 5 weeks , then Measuring survived samples and the length of the growths .

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Using of UV light radiation and BA in the Multiplication stage:

The growths were taken from the previous experiment and for all the used BA concentrations then the explants exposure to UV type C radiation with length 254 nanometer at different periods of UV rays. as shown in (Table 1) at period of time (30, 60, 90 second) and the effect of BA on the vegetative multiplication of buds was studied with concentrations (0.0, 0.25, 0.50 and 0.75) mg / liter and for all explant , with a rate of 6 replications per concentration in glass container . The explants were incubated under the same conditions previously mentioned and measurements were taken after 5 weeks of planting, which included the number of survival and the number of branches and the lengths.

Table (1): UV type C radiation and BA

Groups	**B.A. Cons. Mg / L**	**UV type C Time exposure**	**Replicate**
Groups 1	0.25	30 second	6
Groups 2	0.50		
Groups 3	0.75		
Groups 4	0.25	60 second	6
Groups 5	0.50		
Groups 6	0.75		
Groups 7	0.25		

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Groups 8	0.50	90 second	6
Groups 9	0.75		

Rooting Stage:

The plantings from the multiplication stage were used and transferred to MS supplemented with IBA at concentrations (0.0, 0.3, 0.5 and 1.0) mg / liter with 4 replicates for each concentration then the plantings were incubated under the same previous conditions and measurements were taken after 5 weeks of planting, explant length and survival and roots lengths were calculated as shown in (Table 2).

Table (2): UV type C radiation and IBA

Groups	**IBA. Cons. Mg / L**	**UV type C Time exposure**	**Replicate**
Groups 1	0.25	30 second	4
Groups 2	0.50		
Groups 3	0.75		
Groups 4	0.25	60 second	4
Groups 5	0.50		
Groups 6	0.75		
Groups 7	0.25	90 second	4
Groups 8	0.50		

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Groups 9	0.75		

Acclimatization Stage

The plants were taken then washed well with tap water. Then the plants were immersed in water for one week inside the incubator for the purpose of hardening, considering the daily exchange of water. Then transferred to the sterilized soil, after that planted in the small plastic container with 5 cm diameter. then covered the plastic container and transferred to the acclimatization room under 25° C , 16 hours light and 8 hours dark , then left for 4 weeks with continues irrigation with water contain on Benomyl pesticide with concentration 0.6 gm \ L to ensure the survival of plants. After that creams formed were separated from the plants.

Statistical Analysis:

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study.

Result

1. Initiation stage

The results of the research in the initiation stage of the new growth using the axillary buds of the flower stack showed that it gave the best

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results for the explant used, the results also indicated that the use of the nutrient medium M.S prepared with a B.A 2m/l concentration gave the best growth (fig. 1) , these results are in agreement with (Badea and Sandulescu,2001) showed a different response to tissue culture even though all factors and conditions (nutritional environment and incubation conditions) showed that the gladiolus cultivar showed the best response and its percentage was 80% (Figure 1).

Figure 1: Initiation stage

2. **Multiplication stage**

The result showed after incubation for 5 weeks as shown in the table (3), the plants that continued to grow had the best results using 0.75 and a duration of exposure 30, reaching 6 plants, while the results of the length of the vegetative growth showed that the best growth length appeared using a concentration of 0.50 and an exposure time of 60 reaching 5 cm, as shown in table (3) (fig. 2) in addition to that, the highest average number of branches was shown using a concentration of 0.50 B.A with the duration of exposure 60 reaching 2.6 cm . Note that the number of branches is an indication of the

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number of corms that can be obtained later (Figure 2).

The results achieved by using the B.A and UV, the growth response was shown compared to the control, and this is due to the effect of cytokines in stimulating the lateral shoots on the growth from the dominance to form the branches, without the need to cut off the peripheral bud. Moreover, this may be due to the role of cytokines in increasing the efficiency of the NADH-protochlorophyllid reductase enzyme, which is used in the biological chlorophyll construction and thus increasing its synthesis (Kieber and Schaller, 2018). Metwally *et al.*, (2019) found that UV radiation led to increase in the rate of root length and shoot length. The results were nearly in an ascending order with increasing of UV-B radiation exposure time. The result of Bridgen, (2016) showed at appropriate dosage rates, UV-C light increases branching on some ornamental plants species and increases the number of flowers that are produced.

Table (3): effect of UV and BA on the length and branch number after 40 days on MS media

BA	Time of UV	Survived Samples (6 rp)	Length mean	Branch mean
Control	30	0 ± 0	0 ± 0	0 ± 0
	60	0 ± 0	0 ± 0	0 ± 0
	90	0 ± 0	0 ± 0	0 ± 0
0.25	30	5 ± 0.12	2.5 ± 0.03	1.4 ± 0.05
	60	4 ± 0.09	3.5 ± 0.05	1.5 ± 0.02
	90	2 ± 0.04	1.5 ± 0.02	1.0 ± 0.02
0.50	30	5 ± 0.12	4 ± 0.09	2.0 ± 0.04
	60	5 ± 0.12	5 ± 0.12	2.6 ± 0.03
	90	2 ± 0.04	2 ± 0.04	2.0 ± 0.04
0.75	30	6 ± 0.19	3 ± 0.07	1.66 ± 0.02
	60	4 ± 0.09	4 ± 0.09	1.75 ± 0.02
	90	2 ± 0.04	2 ± 0.04	2.0 ± 0.04
LSD value		1.065 *	1.173 *	0.588 *
* (P≤0.05).				

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Figure 2: Branch lengths were obtained from 60-sec exposure time at conc. 0.50 for BA.

Rooting stage

The result of table (4) showed the use of different concentrations form IBA and UV. That the effect of IBA and UV played a role in the survival rate of the plants as well as the growth and root length of the growing plants as the highest percentage of plant survival was using concentrate 0.50 IBA and duration of exposure is 30 seconds, reaching 4 plants, and it did not differ significantly from concentration 1 and exposure period of 30 seconds. In addition, the best length of plant growth was using the concentration 0.50 IBA exposure periods of 60 seconds where it reached 6 cm (fig. 3). In addition, a root length reach to 2.5 cm at the same concentrations of 0.50 IBA exposure periods of 60 seconds which not differs significantly from the length of the roots using 0.25 IBA exposure periods of 60 seconds and the concentrations of 1 IBA exposure periods of 60 seconds. The results related to the rooting stage showed that the IBA and UV. had a role in the above studied traits, as IBA stimulates cells to divide and form roots, as well as the UV. , a study of (Ahandani *et al.*, 2019) shows that *Dracocephalum* plants are sensitive to UV-C and UV-A with trend to UV-C and this finding give an insight into the physiological and morphological changes on root and leaf

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after 6 week of UV exposure and indicate the sensitivity of these plants to UV-C more than UV-A radiation (Figure 3).

Table (4): effect of UV light and IBA on the length and root length after 35 days on MS media

IBA	Time of UV	Survived Samples (4rp)	Length mean	Root length mean
Control	30	0 ± 0	0 ± 0	0 ± 0
	60	0 ± 0	0 ± 0	0 ± 0
	90	0 ± 0	0 ± 0	0 ± 0
0.25	30	3 ± 0.07	3 ± 0.07	1.5 ± 0.02
	60	2 ± 0.04	4 ± 0.08	2.5 ± 0.06
	90	2 ± 0.04	2.5 ± 0.06	1.5 ± 0.02
0.50	30	4 ± 0.08	4.5 ± 0.11	1.5 ± 0.02
	60	3 ± 0.07	6 ± 0.15	2.5 ± 0.06
	90	2 ± 0.04	3 ± 0.07	1.5 ± 0.02
1	30	4 ± 0.08	3.5 ± 0.07	1.5 ± 0.02
	60	3 ± 0.07	4.5 ± 0.11	2.5 ± 0.02
	90	2 ± 0.04	3 ± 0.07	1.5 ± 0.02
LSD value		1.038 *	1.461 *	0.744 *
* ($P \leq 0.01$).				

Figure 3: Root lengths were obtained from 60 sec exposure time at conc. 0.50 for BA.

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