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# MICROPROPAGATION AND BULBLET FORMATION *In vitro* OF DUTCH *Iris* cv. BLUE MAGIC

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## ABSTRACT

*The in vitro growth and development of Dutch Iris cv. Blue Magic plants is determined by a number of complex factors including nutrients, sucrose and plant growth regulators . The effect of cytokinins ( BA ) and auxin ( NAA ) as well as type of medium (MS, B5 and WPM )on in vitro growth were studied multiplication stage while plant growth retardants ( CCC,PP333 and ABA) and sucrose were studied too during bulb formation stage. The effect of BA and NAA combinations on the production of Dutch Iris cv. Blue Magic bulbs from shoots in vitro was investigated . Combinations between BA at 1.0or 2.0mg /l and NAA at 0.2 mg/l were the best medium for shoot formation estimated as number of shoot, shoot length, number of leaves and fresh weight. Shoot formation of Iris was affected by MS, B5 and WPM media. Plantlets cultured on MS medium recorded the best shoot formation for shoot formation followed by B5 and WPM media. At bulblet formation , the highest number of bulbs and bulbs percentage were only obtained with the medium contained CCC at 25 µM, while the highest number of shoots and leaves were recorded when the medium contained 5,10,20,and 25 µM ABA compared to other treatments. The effect of sucrose concentrations (30, 60 and 90 g/l) on blublet formation were studied. MS nutrient media containing 25 µM $PP_{333}$ and 90g/l sucrose increased significantly the average number of bulbs/shoot and diameter of bulbs. Plantlets derived from 90g/l sucrose containing 25 µM $PP_{333}$ showed the highest percentage of survival (90%) at acclimatization stage. The developed shoots were transferred to a rooting medium containing two types of auxins ( IBA or NAA each at 0.0, 1.0, 2.0 and 3.0 mg/l ). The treatment of MS medium containing 2 mg/L NAA produced the largest number of roots as well as root length. Plantlets grown on MS rooting medium supplemented with 2.0mg/l NAA showed the highest percentage of survival (85%)during acclimatization stage. In this work, the soil mixture used in acclimatization consisted of peatmoss : perlite (2:1v/v).*

**Key words:** *In vitro* -Dutch *Iris* cv. Blue Magic- bulbs- growth retardants.

## INTRODUCTION

The genus *Iris* includes over 300 species. Many of which are valuable and horticulturally important. Flowers are either bearded or beardless types and are composed of parts in sets of three. The species may differ in shoot structure. Some

being bulbous, some rhizomatous and a few stoloniferous (Lenz 1978). Like other ornamental monocotyledonous species with bulbs or rhizomes, irises are generally propagation vegetatively. *Iris* Blue Magic standards campanula violet with deeper blue falls white edged yellow blotch; the most cold resistant Dutch *Iris*. (10+cm). Often blooming around 'Mother's Day', these are good, long-lasting cut flowers and are ones that make nice clumps in the late spring garden; rather formal flowers that give wonderful linear form to the border 18-20 zones 6-9. Bulb size 8+cm noted otherwise. Many studies have appeared regarding *in vitro* propagation of some *Iris* species using shoot apices (Reuther,1977), rhizomes (Kromer,1985), young inflorescences (Meier *et al*.,1975), mature emberyos (Stoltz, 1977), and bulb scales (van der Linde *et al*.1988). Rapid propagation is desirable to multiply new varieties. The technique of tissue culture for speeding up propagation has been applied widely in garden species (Mantell *et al*., 1985) and the potential of this technique has been clarified in the genus *Iris:* Fujino (1972) and Hussey (1976) for Dutch *Iris,* I. *hollandica* Hort.; Meyer *et al*.(1975) for tall bearded irises, *Iris germanica* L.; (Randojevic *et al.*,1987) for *Iris pumila* L. and so on. However, the *in vitro* propagation of *Iris ensata* remains an unsolved problem, although plant regeneration by means of flower organ culture was reported (Ichihashi and Kato,1986; Kawase *et al*., 1991). Yabuya *et al*.(1991) reported that produce 1,000 plantlets, therefore, ca. 11 scapes are required for the culture. Thus, this *in vitro* technique has considerable potential to speed up the propagation of garden varieties in *Iris ensata*. Further improvements in this technique are, however, needed to establish methods for a large scale propagation.

The present study aims to standardize a technique for *in vitro* propagation and bulbelet formation of *Iris* Blue Magic.

## MATERIALS AND METHODS

This work was conducted in the Tissue Culture Laboratory, Genetic Engineering and Biotechnology Research Institute (GEBRI), Menofyia University, Sadat City, during the period from 2004 to 2006

Surface sterilization of Bulbs-scale explants

Iris bulbs as a plant material were imported from Holland.

The Bulbs-scale of *Iris* were rinsed in running tap water for 30 minutes, dipped in 70 % ethanol for 10 seconds. The explants, were soaked in chlorox solution at 3% NaOCl concentration with one drop of tween 20 (Polyoxyethlene sorbitan monolaurate)- as a wetting agent -per 100 ml of sterilizing solution for 10 minutes. After sterilization, the explants were rinsed three times in autoclaved distilled water to remove all traces of the disinfectant. Bulbs-scale explants were cultured *in vitro* on MS medium supplemented with 1 mg/1 NAA (α-naphthaleneacetic acid), 1 mg/16-BA(6- benzyladenine), 30 g/l sucrose and 6 g/l agar. The pH of the medium was adjusted to 5.8 prior to autoclaving (Yabuya *et al.*,1991). The medium was poured into the culture jars (325 ml) where each jar contained 50 ml of the medium. The jars were capped with polypropylene closures and autoclaved at 121 C$^{o}$ and 1.2 kg /cm$^{2}$ air pressure for 20 minutes.

**Multiplication stage**

***Effect of BA and NAA at different concentrations on shoot proliferation***

In order to achieve shoot proliferation, materials (shoots of about 2cm long with 1-2 leaves) obtained from the previous stage were cultured on MS medium supplemented with different concentrations of BA (0.0, 1.0, 2.0 and 3.0mg/l) and NAA (0.0, 0.10, 0.20 and 0.30mg/l) each alone and all possible combinations, leading to 16 treatments.

***Effect of type of media on in vitro growth.***

This experiment was carried out to study the effect of media type (MS ,B5 and WPM salt strength) on growth and development of *Iris* cultured *in vitro* (multiplication stage) and this experiment was repeated twice. Three shoots -at length of 2 cm produced from the multiplication stage (MS +0.2mg/l NAA+2.0mg/l BA+30g/L sucrose) - were cultured in each jar (325 ml) which contained 50 ml of the following media MS ( Murashige and Skoog, 1962), B5 (Gamborg, 1968) or WPM (McCown and Lloyd, 1980). Each treatment contained 10 replicates (jars) and each replicate had 3 explants (shoots). After three subcultures (four weeks / subculture ) data were recorded as number of shoots, number of leaves, shoot length and fresh weight (g) / culture (jar).

**Bulbs formation stage**

***Effect of plant growth retardant***

In this stage, the effect of plant growth retardant on bulb formation were studied. Shoots produced from multiplication stage(MS medium supplemented with 1mg/l BA + 0.2 mg/l NAA) were transferred to MS basal medium free from plant growth regulator and incubated for four weeks. The obtained shoots( 3cm long ) were cultured on MS medium supplemented with cycocyl (CCC), paclobtrazol ($PP_{333}$) and abscisic acid (ABA) at different concentrations (0.0, 5,10,15,20 and 25µM ).

***Effect of sucrose***

Shoots produced from paclobtrazol at 25 µM were cultured on MS medium supplemented with sucrose at 30, 60 and 90 g/l. Each treatment contained 10 replicates (jars). Each jar had five shoots. After six weeks of incubation, data were recorded as number of shoot, number of leaves, shoot length (cm), number of bulblet, bulblet percentage and fresh weight (g) / culture. The cultures were incubated at 15 ºC day and night (van der Linde *et al*. 1988). Light was provided by white fluorescent tubes giving light intensity 2000 lux at the level of explants for 16 hours per day.

**Rooting stage**

***Effect of auxin***

After multiplication stage, shoots obtained from multiplication medium (MS+ 1.0mg/l BA and 0.2mg/l NAA) were cultured in MS medium with indolyl-3-butyric acid (IBA) or α-naphthalene acetic acid (NAA) at 0.0, 1.0 or 2.0 mg/l. Ten replicate jars with three shoots per jar were used for each treatments. The cultures were incubated at 20ºC day and night. Light was provided by white fluorescent tubes giving light intensity 2000 lux at the level of explants for 16 hours per day.

**Acclimatization stage:**

In order to transfer the previously obtained plantlets form *in vitro* status (jars) to the greenhouse conditions, plantlets were transferred to plastic pots ( 6 cm diameter) contained mixture of peatmoss and perlite (2:1,v:v), then, the plantlets were kept in greenhouse and covered with a plastic bag. The effect of the used axins (during rooting stage) on survival percentage at acclimatization stage was studied. The percentage of survival was calculated after four weeks of acclimatization.

According to experimental design, *in vitro* experiment was conducted to examine the effect of different concentrations of sucrose (30, 60, and 90 gm/l) containing paclobtrazol at 25 µM on bulbs formation and growth of plantlets. The number of bulbs were recorded after 6 weeks of incubation *in vitro*. The effect of sucrose concentration on the percentage of survival at acclimatization stage was also studied the produced plantlets were grouped according to the treatments used at the last experiment. Those plantlets were transferred to the same mixture that mentioned above and the same greenhouse conditions. Data were recorded as the survival percentage in each group (treatment).

The effect of auxin ( IBA and NAA at 1, 2, and 3 mg/l) containing rooting media on survival percentage during acclimatization stage were studied too in the greenhouse.

The randomized factorial design was used and data were subjected to analysis of variance. Separation of means among treatments was determined using LSD test at 5% (Steel and Torrie, 1980).

## RESULTS AND DISCUSSION

**Shoot proliferation:**

***Effect of BA and NAA at different concentrations on shoot proliferation:***

Results in Table 1 clearly show the effect of BA and NAA on shoot proliferation estimated as number of shoot, number of leaves, shoot length and fresh weight. Results showed that concentration of BA at 1.0and 2.0mg/l was more effective (19.17 and 21.58 respectively ) than medium free hormone and high concentration BA at 3mg/l (12.67 and17.25 respectively) in shoot proliferation. Results on the main effect of NAA indicated that the presence of NAA at the concentration 0.1 and 0.2 mg/l showed a significant effect on shoot number, the concentration 0.3mg/l negatively affected the number of obtained shoots. The original data (interaction between BA and NAA) showed that the addition of BA alone to the medium showed lower effect on shoot proliferation compared to the high effects of the other treatments. Similar effect of BA alone was significantly obtained with medium free hormone (control). The highest shoot number/culture (31 and 27) was obtained with the medium contained 1.0 and 2.0mg/l BA combined with 0.2mg/l NAA as shown in Figure 1. Although, the addition of NAA to the medium alone showed low effect on shoot number compared to the highest effect mentioned above, but all used concentrations of BA alone significantly surpassed the control.

Concerning the number of the produced leaves, data on the main effect of NAA indicated that the presence of NAA at the concentration 0.3 mg/l significantly increased the number of leaves/culture (45.5) compared to the control and other concentrations. As for the main effect of BA on number of leaves, data show that the highest and similar responses were significantly found with the concentration 1.0 and 2.0mg/l. However, lower responses significantly obtained with the concentration 3.0mg/l and control. Regarding the interaction, the highest number of leaves/culture (80.67) was obtained with the treatment contained 2mg/l BA and 0.3 mg/l NAA compared with other combination treatments. However, both treatments significantly surpassed all other treatment including the control (12.67).

As for the main effect of BA on the shoot length, data in the same Table 1 indicate that the concentration 1.0 and 2.0 mg /l significantly produced the highest response. Lower responses were obtained with high levels of BA 3mg/l and control. The original data revealed that the highest value of shoot lengths/culture was obtained with medium contained 2.0mg/l BA and other concentration of NAA without significant difference.

Regarding the fresh weight, data in Table 1 indicated that BA concentration significantly increased fresh weight. The highest fresh weight was obtained by 1,2,3 mg/l BA (3.82,4.21, 3.53 mg/l respectively) followed by control. There is not significant effect of NAA at different concentration on fresh weight. The interaction was significant at 5% level.

These results go in line with these obtained by van der Linde *et al*.(1988) found that the basal part of the shoot and growing *Iris* it on MS basal medium containing 0.1mg/NAA and BA / litre at 15 C° resulted in a multiplication factor of four with a seven week cycle. Explants of young scrapes of *Iris ensata* were cultured on MS medium with 1 mg/1 NAA, 1 mg/1 6-BA, 30 g/1sucrose and 10 g/1 agar, and this species was characterized by high variety specificity for callus, shoot and root induction (Yabuya. *et al*.1991).

**Effect of type of medium**

*In vitro* shoot number of *Iris* was affected by type of media (MS, B5 and WPM). MS medium was more effective than B5 and WPM as illustrated in Figures 1 and 2. The best number of shoot was recorded in MS medium (44.33) while the lowest was recorded with WPM (18.67). The highest number of leave was obtained with MS media (54.33) compared with B5 and WPM media (41 and 30.33, respectively).

Data also showed that shoot length was affected by media type stimulated shoot formation comparing to MS as presented in Figure1 and Photo 2. MS media recorded the best results for shoot length (14 cm). *Iris* fresh weight was affected by MS,B5 and WPM treatments. Plantlets cultured on MS medium recorded the best result for fresh weight (12.77 g/culture) followed by B5 and WPM media as presented

**Table 1 :Effect of BA and NAA concentration on shoot proliferation of *Iris in vitro***

| BA mg/l | NAA mg/l | | | | |
|---|---|---|---|---|---|
| | **0.0** | **0.10** | **0.20** | **0.30** | **Mean (A)** |
| | | | **Number of shoot** | | |
| **0** | 6.00 | 15.67 | 14.33 | 14.67 | 12.67 (c) |
| **1** | 6.00 | 23.67 | 31.00 | 16.00 | 19.17 (ab) |
| **2** | 13.33 | 23.67 | 27.00 | 22.33 | 21.58 (a) |
| **3** | 16.67 | 18.00 | 18.67 | 15.67 | 17.25 (b) |
| **Mean (B)** | **10.50 (c)** | **20.25 (a)** | **22.75 (a)** | **17.16 (b)** | **LSD 5% Level AxB: 5.81** |
| | | | **Number of leave** | | |
| **0** | 12.67 | 21.00 | 22.33 | 32.00 | 22.00 (c) |
| **1** | 36.67 | 35.00 | 40.67 | 35.33 | 36.42 (ab) |
| **2** | 35.67 | 30.67 | 40.67 | 80.67 | 46.92 (a) |
| **3** | 29.00 | 47.00 | 31.00 | 34.00 | 35.25 (b) |
| **Mean (B)** | **28.50 (b)** | **33.42 (b)** | **33.67 (b)** | **45.50 (a)** | **LSD 5% Level AxB: 21.42** |
| | | | **Shoot length** | | |
| **0** | 4.33 | 4.00 | 11.67 | 4.33 | 6.08 (b) |
| **1** | 7.67 | 14.67 | 11.00 | 11.33 | 11.17 (a) |
| **2** | 12.33 | 12.00 | 10.00 | 10.67 | 11.25 (a) |
| **3** | 9.33 | 5.33 | 7.33 | 8.00 | 7.50 (b) |
| **Mean (B)** | **8.42(a)** | **9.00 (a)** | **10.00(a)** | **8.58 (a)** | **LSD 5% Level AxB: 3.42** |
| | | | **Fresh weight (g)** | | |
| **0** | 2.15 | 2.71 | 4.12 | 2.80 | 2.95 (b) |
| **1** | 3.05 | 4.49 | 4.56 | 3.17 | 3.82 (a) |
| **2** | 4.84 | 3.55 | 4.50 | 3.94 | 4.21 (a) |
| **3** | 3.62 | 3.66 | 3.07 | 3.75 | 3.53 (ab) |
| **Mean (B)** | **3.42 (a)** | **3.60 (a)** | **4.06 (a)** | **3.41 (a)** | **LSD 5% Level AxB: 1.553** |

in Figue 1. WPM medium recorded the lowest values which recorded 6.6 g/culture. Statistically analysis showed significant difference amongst treatments at 5% level. These results are in harmony with (Rungini and Verma, 1983); (Caboni, 1994) they found that MS medium gave the highest shoot multiplication rate and growth of almond.

For shoots obtained from the scape culture, effects of sucrose concentrations and activated charcoal on root induction were examined by using 1/2 MS with 1 mg/l NAA, 1 mg/1 6-BA, 30 g/1 sucrose and 10 g/l agar as the basic medium. (Naik and Nayak 2005).

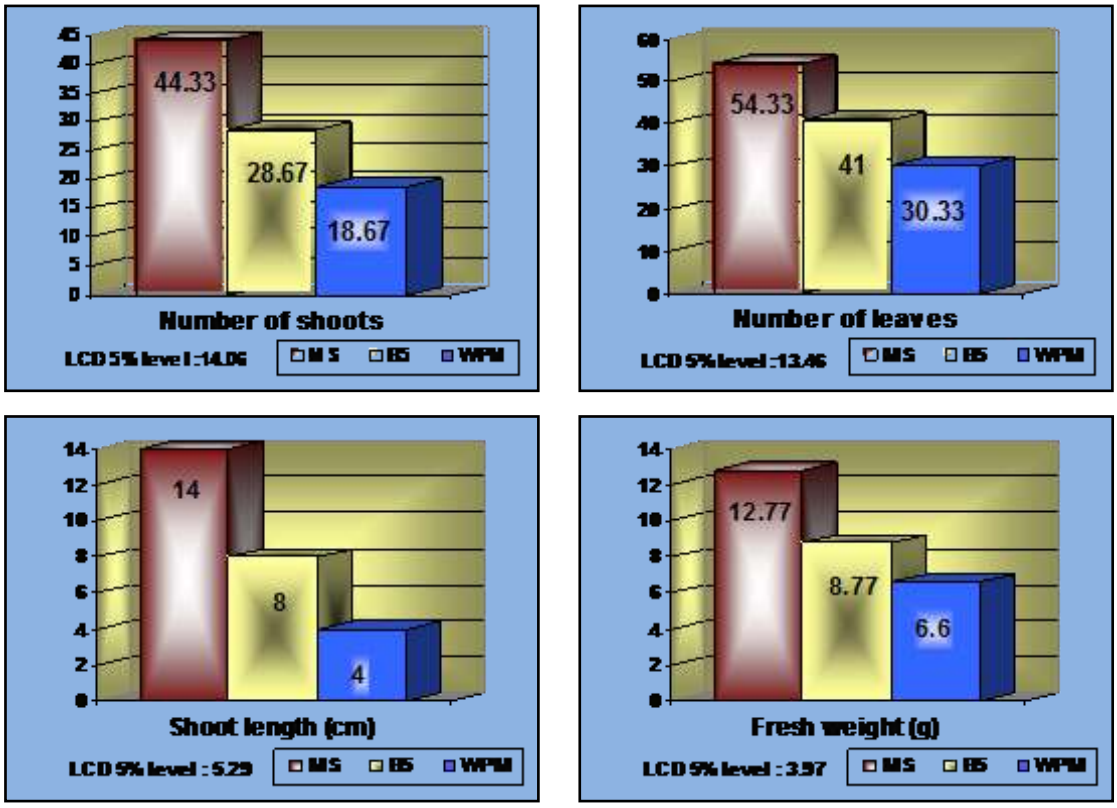


**Figure 1 :Effect of media type on shoot proliferation of Dutch *Iris in vitro*.**

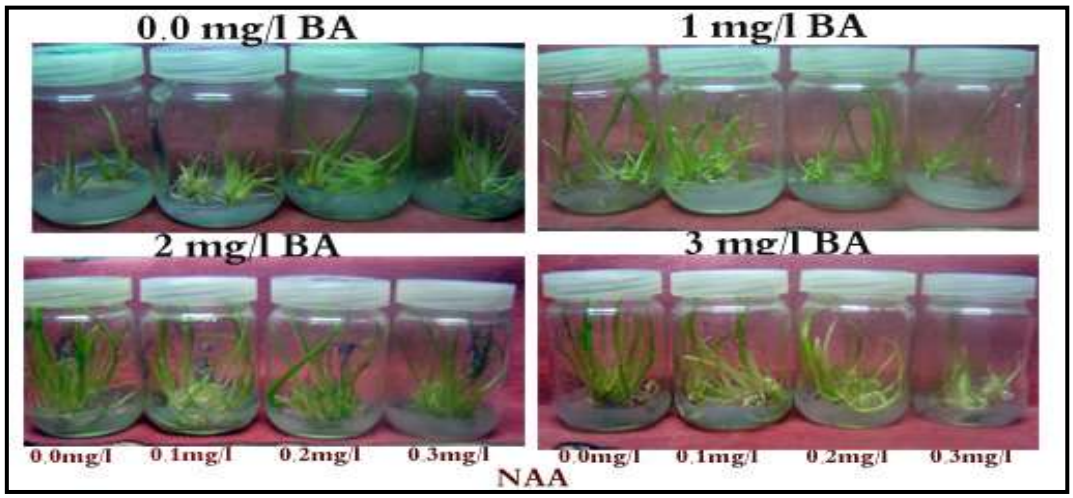


**Photo 1 :Effect of BA and NAA concentration on shoot proliferation of Dutch *Iris.***

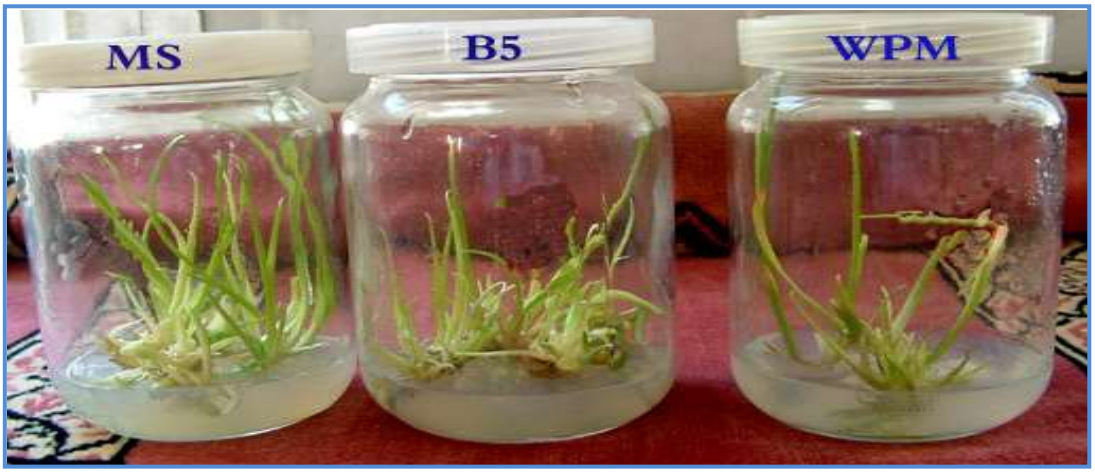


**Photo 2:Effect of media type on shoot proliferation of Dutch *Iris in vitro*.**

**Bulbs formation**

***Effect of some growth retardant on bulblets formation of Dutch Iris cultured in vitro after three months.***

In this experiment the effect of some growth retardants (CCC, $PP_{333}$ and ABA) on blublet formation were studied. Shoot length was affected by growth retardants (CCC, $PP_{333}$ and ABA) and using MS medium without any growth retardants (control) significantly increased the average shoot length (15.00 cm) compared with other treatments as presented in Figure 2 and Photo 3. MS nutrient media containing ABA at 5 and 10 µM significantly increased the number of shoots (47.33 and 46.33 respectively ) and number of leaves (58.33 and 58.67 respectively) compared to other treatments. Number of bulbs and bulb percentage was affected by growth retardants, MS medium containing CCC at 25 µM increased significantly the average number of bulbs and bulb percentage (29 and 83 %, respectively) compared with other treatments. Adding CCC at 10µM to culture medium gave the highest significant fresh weight (15.47g) compared with other treatments. Paclobutrazol at all concentrations and control recorded the lowest values of the fresh weight, which recorded 7.11, 9.03, 7.51, 8.22 and 5.00 g/culture, respectively. Statistically analysis showed significant difference among the treatments at 5% level.

These results go in line with these obtained by Kim and Han (1993) found that the percentage of cormlet formation was the highest by 65.8%in primary liquid medium with 10µMCCC.Goo and Kim (1994) reported that the media with the addition of 10µM ABA to 9% sucrose concentration , the days for cormlet formation was shortened to 46.1 days , but the percentage of cormlet formation ,the numbers of cormlet per shoot and fresh weight per cormlet was decreased of *Gladiolus* cv. Topaz *in vitro*. Paclobutrazol shifted assimilate allocation preferentially towards cormel development of *gladiolus* (Steinitz *et al*., 1991). Paclobutrazol created and maintained the conditions required for continuous cormel growth of *gladiolus* (Ziv 1989, Steinitz and Lilien-kipnis, 1989 and Steinitz *et al*., 1991)

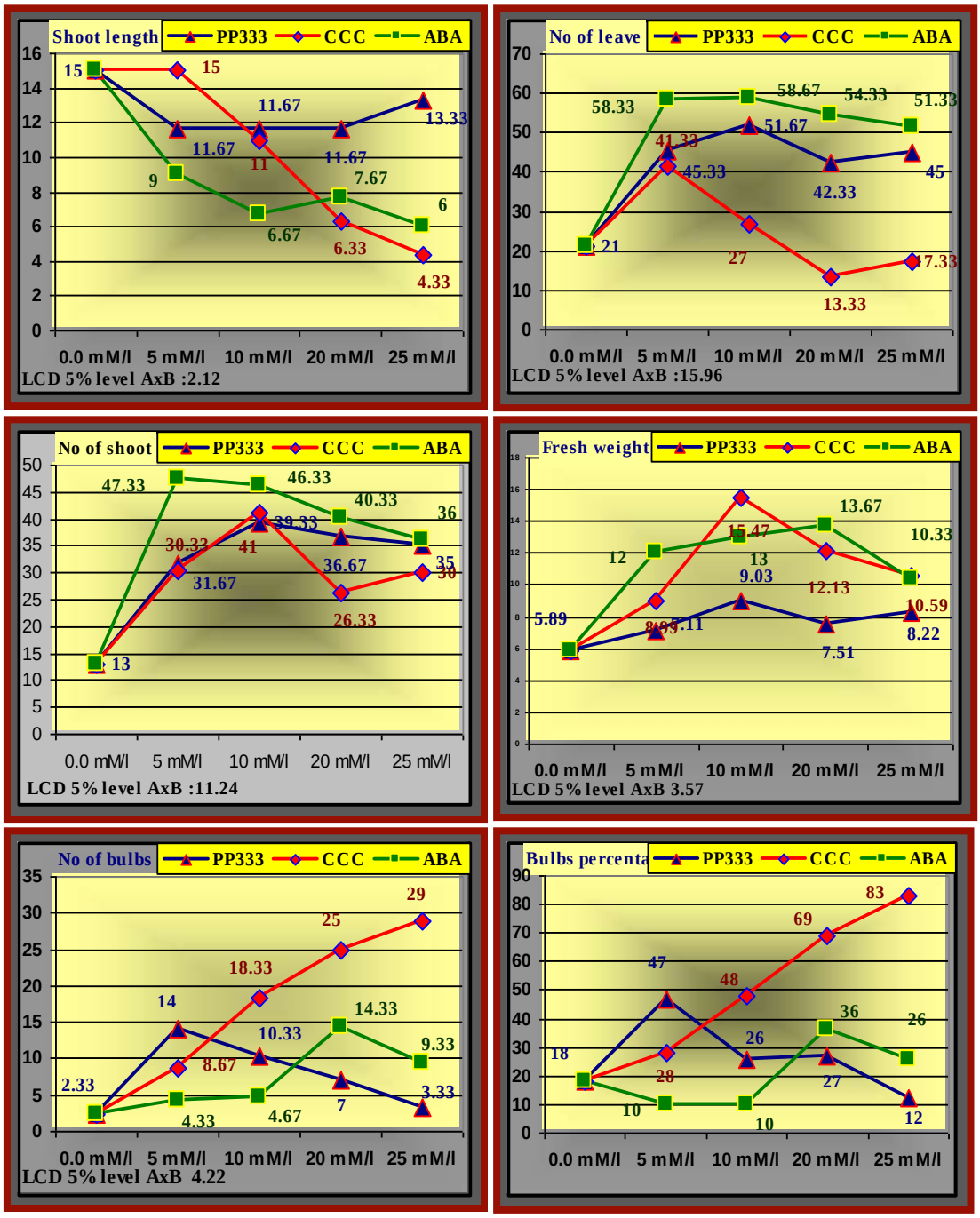


**Figure 2 :Effect of growth retardant type on different parameter of *Iris in vitro*.**

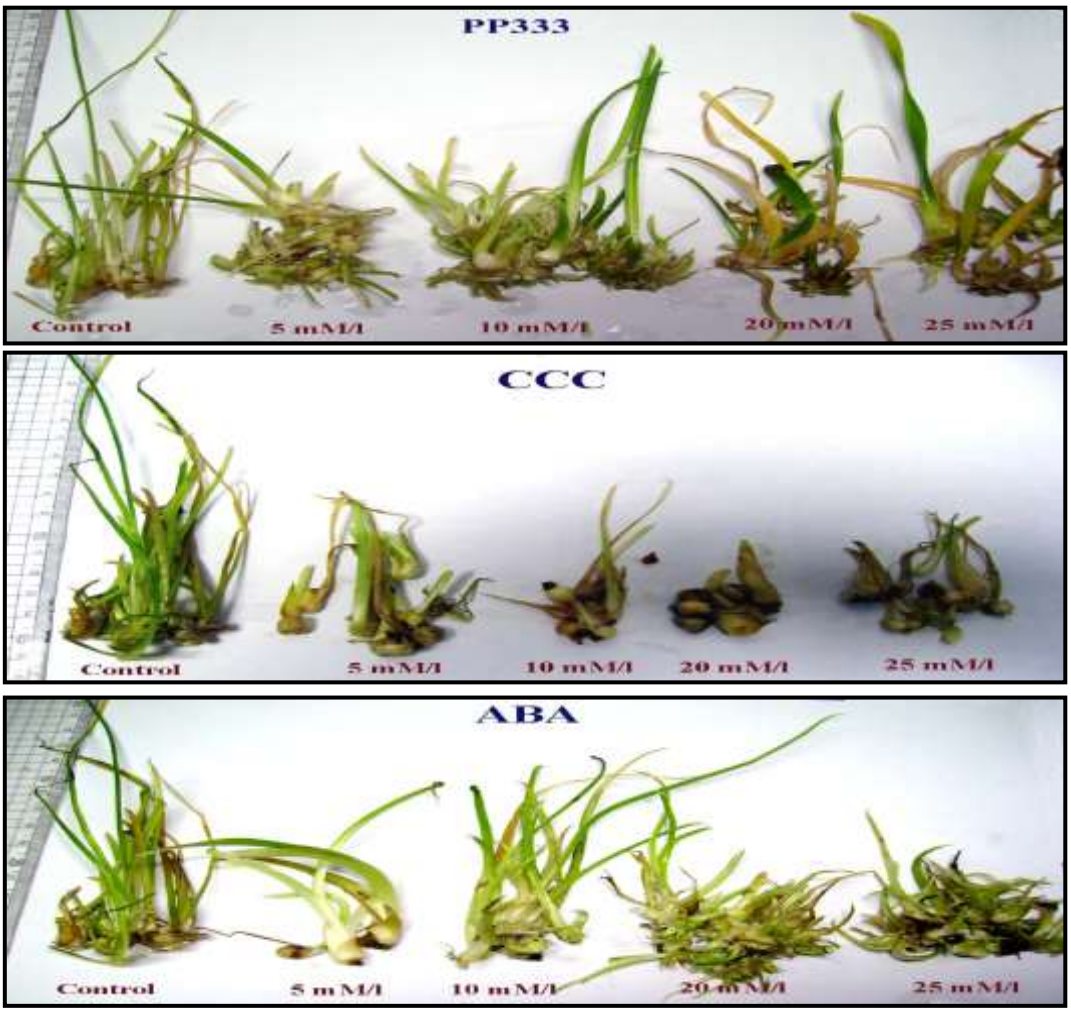


**Photo 3: Effect of some growth retardant on bulblets formation of Dutch *Iris* cultured *in vitro* after three months.**

***Effect of sucrose concentration on bulblets formation of Dutch Iris cultured in vitro after three months:***

In this experiment the effect of sucrose concentrations (30, 60 and 90 g/L) on blublet formation were studied as presented in Table 2 and Photo 4.

Shoot length was affected by sucrose concentrations (30, 60 and 90 mg/ L) using MS medium with 30 g/L sucrose significantly increased the average shoot length, number of leaves and number of shoots (11.80,11.20 and 12.00 cm, respectively) compared with other treatments. MS medium containing 60g/ sucrose significantly increased the root number and length (5.60 and 2.60, respectively) compared with 30 and 90 g/l sucrose. Number of bulbs and diameter of bulbs were affected by sucrose concentrations. MS nutrient medium containing sucrose at 90g/ L increased significantly the average number of bulbs/shoot and diameter of bulbs (3

and 0.70 cm, respectively) compared with 30 and 90 g/L sucrose. Statistically analysis showed significant difference among the treatments at 5% level.

These results go in line with these obtained by (Nagaraju *et al*., 2002 ). found that the large-scale multiplication of true to type planting materials of selected varieties/ hybrids and the commercialization of newly released hybrids *gladiolus*, supplementation with 10 mg/L paclobutrazol and 60–120 g/L sucrose, according to variety/species/ hybrid is optimum MS basal medium supplemented with NAA (1 mg/L), BA (2mg/L) and sucrose 60 g/L was found to be most effective in inducing 12-15 bulblets of different sizes (2-10mm) within 4-5 weeks. Bulblets were induced at the base of the regenerants upon transfer to MS basal medium supplemented with enhanced concentrations of sucrose at 45 to 90 g/L. Larger tubers were obtained on media containing 6-9%, compared to 3 or 12% sucrose. The highest mean fresh weight of primary tubers was on explants of *Gloriosa rothschildiana* on medium with half-MS and 6% sucrose, but for the growth of secondary tubers, the medium containing half-MS and 9% sucrose was most successful (Kozak, 2002). Paclobutrazol and sucrose levels in the media were found to significantly affect starch accumulation, growth value and dry weight percentage of liquid-cultured meristematic clusters of daylily (Chen *et al.*, 2007).

**Table 2 :Effect of sucrose concentration on growth and development of Dutch *Iris* cultured *in vitro* after three months.**

| Sucrose g/l | Shoot formation | | | Root formation | | Bulbs formation | |
|---|---|---|---|---|---|---|---|
| | Shoot length | No. of leaves | No. of shoots | No. of roots | Root length | No of bulbs/ shoot | Diameter of bulbs (Cm) |
| **30** | 11.80 (a) | 11.20 (a) | 12.0(a) | 2.00 (c) | 0.36 (c) | 0.00(c) | 0.00 (c) |
| **60** | 9.60 (b) | 6.40 (b) | 7.20 (b) | 5.60 (a) | 2.60 (a) | 1.60(b) | 0.38 (b) |
| **90** | 5.80 (c) | 4.20 (b) | 3.80 (c) | 3.80 (b) | 1.70 (b) | 3.00(a) | 0.70 (a) |
| **L.S.D** | **1.726** | **2.337** | **1.756** | **1.291** | **0.490** | **0.754** | **0.153** |

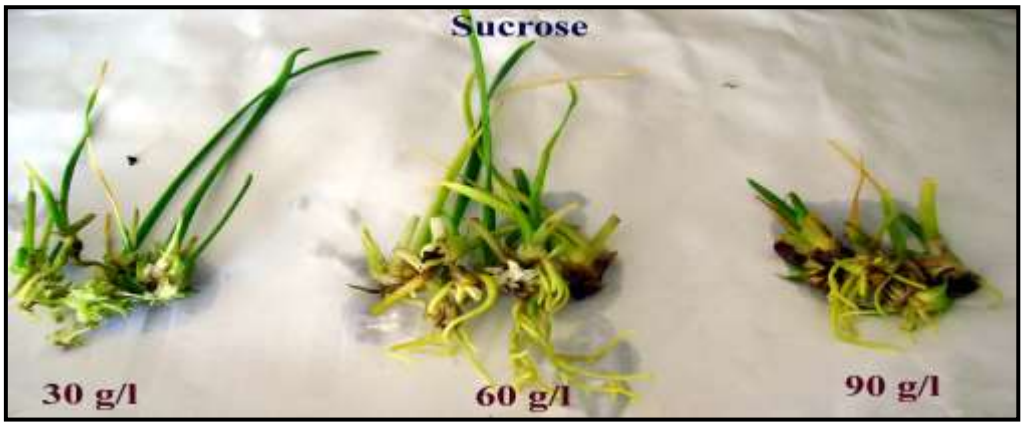


**Photo 4: Effect of sucrose concentration on growth and development of Dutch *Iris* cultured *in vitro* after three months.**

**Root formation**

***Effect of auxin type and concentrations on growth and development of Dutch Iris cultured in rooting stage.***

In this experiment the effect of auxine type (IBA and NAA) and concentration (1, 2 and 3 mg/ L) on root formation were studied and presented in Table 3 and Photo 5. The effect of auxine concentrations, was observed where, IBA at 1 or 2.0 mg/L resulted in the highest plant and root length in Table 3. Results revealed that, the greatest number of roots was obtained on MS medium supplemented with 3 mg/ L NAA (17.00) than other treatments. The original data show that the root percentage (90%) was a feature of culture grown on a medium contained 1 and 2 mg/ L NAA.

These results go in line with these obtained by Gupta and Sehgal (1997) who found that all media supplemented with 10uM NAA recorded the highest rooting percentage (96.67 %) and the highest root number were achieved on half-strength MS medium with 0.8 % agar and 3.0 % sucrose of *Gladiolus* cv. sylvia. Lilien and Kochba (1987) found that for *Gladiolus cr. Kineret*, maximum number of roots developed at 3 uM NAA. Root length decreased, root diameter increased and root weight per plantlet also increased with rising concentration of NAA. Mercer and Kerbauy (1992) showed that addition of 0.54 μM NAA was necessary to stop adventitious proliferation as well as to reestablish apical growth of the shoots of *Vriesea Fosteriana* when shoots attained ca. 2cm in high (3 months of culture), rooting was easily induced, and the shoots were transferred to medium supplemented with 1.1 μM NAA. After 2 months of culture every shoot formed 3-4 roots.

**Table 3 :Effect of auxin type and concentration on growth and development of Dutch *Iris* cultured in rooting stage.**

| Auxin type | Auxin conc. (mg/l) | Shoot length (cm) | No of leaves | No of roots | Root length (cm) | Rooting % |
|---|---|---|---|---|---|---|
| **Control** | | 11.60 (cd) | 6.00 (a) | 0.40 (e) | 0.32 (c) | 10.00 |
| **IBA** | **1.0** | 19.20 (a) | 4.80 (bc) | 1.20 (e) | 3.00 (a) | 17.00 |
| | **2.0** | 17.20 (ab) | 5.40 (ab) | 4.20 (d) | 1.70 (b) | 40.50 |
| | **3.0** | 13.20 (c) | 4.20 (c) | 6.60 (c) | 0.50 (c) | 60.50 |
| **NAA** | **1.0** | 13.20 (bc) | 5.40 (ab) | 7.20 (c) | 1.90 (b) | 90.00 |
| | **2.0** | 14.40 (bc) | 4.60 (ac) | 17.00 (a) | 0.80 (c) | 90.00 |
| | **3.0** | 8.80 (d) | 2.60 (d) | 9.90 | 0.28 (c) | 70.00 |
| **L.S.D at level 5%** | | **2.996** | **0.991** | **1.989** | **0.722** | |

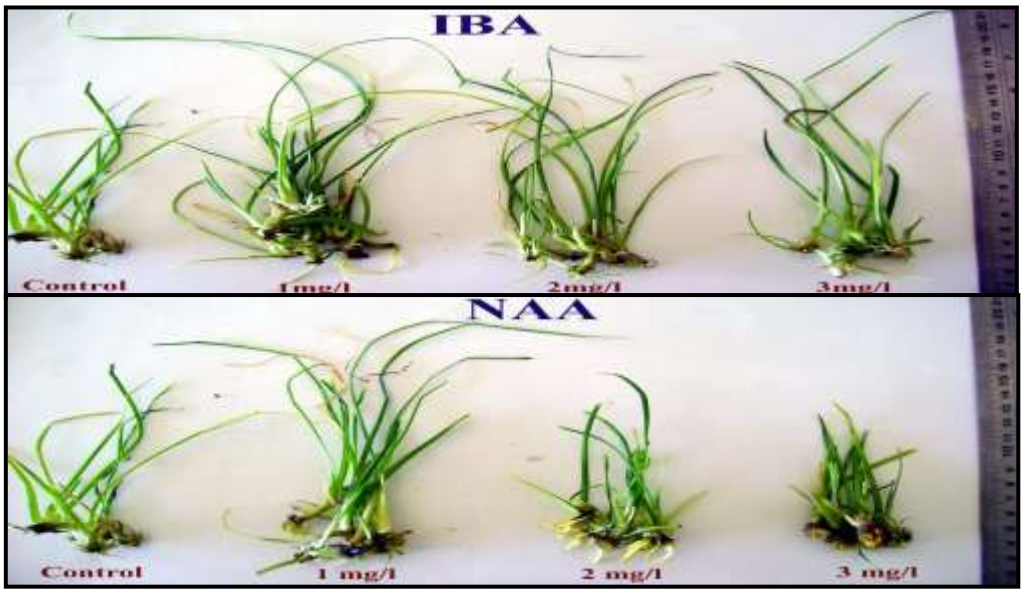


**Figure 5: Effect of auxin type and concentration on growth and development of Dutch *Iris* cultured in rooting stage.**

Plantlets were transferred to the soil mixture in separated groups according to treatments of the previous experiment of sucrose concentration. Data in Table 4 indicated that plantlets derived from 90g/L sucrose containing 25 µM PP333 showed the highest percentage of survival (90 %) at acclimatization stage (Photo 6). The highest response was followed by survival percentage of plantlets obtained from 60 g/ L sucrose (80 %). While, the groups of plantlets obtained from the other treatments showed lower percentages of survival, the lowest percentage (35%) was obtained with plantlets derived from the 30g/ L sucrose.

**Table 4: Effect of sucrose concentration used during *in vitro*, on the survival rate of Dutch *Iris* plantlets at acclimatization stage.**

| Sucrose concentration | Survival percentage at acclimatization |
|---|---|
| **30 g/l** | 35 |
| **60 g/l** | 80 |
| **90 g/l** | 90 |

This experiment was conducted in rooting stage by transferring plantlets grown *in vitro,* directly to the soil mixture. In Table 5, data indicated that the plantlets produced from MS rooting medium( without auxin ) recorded the lowest survival percentage(10%) during acclimatization stage. The gradual increase in IBA and NAA concentration in rooting stage resulted in gradual increase in the percentage of survival at acclimatization. The greatest percentage of survival (85%) in greenhouse was obtained when the shoots cultured in rooting medium supplemented with 1and 2 mg/L NAA.


**Photo 6 : Effect of sucrose concentration used during *in vitro*, on the survival rate of *Iris* plantlets at acclimatization stage.**

**Table 5: Effect of IBA and NAA used during *in vitro*, on the survival rate of *Iris* plantlets at acclimatization stage.**

| Auxin tupe | concentration | Survival percentage at acclimatization |
|---|---|---|
| **Control** | | 10 |
| **IBA** | **1mg/l** | 40 |
| | **2 mg/l** | 40 |
| | **3 mg/l** | 60 |
| **IAA** | **1 mg/l** | 85 |
| | **2 mg/l** | 85 |
| | **3 mg/l** | 55 |

These results are in line with these obtained by Dimech *et al.* (2007) who found that regenerated shoots were rooted of Gymea Lily *ex vitro* after 6 weeks when dipped in a solution of 50 µM NAA. Naik and Nayak (2005) reported that transplantation of Bulblets into soil. All *in vitro* plantlets with bulbs induced in culture showed a survival rate of about 80% when transferred to the potted soil. But, survival rates of bulblets produced directly on explants varied for different size of bulblets. Small bulblets (2-3 mm diam.) showed a survival rate of 40-50%, whereas, the larger bulblet (4-10 mm diam.) had a 70-80% survival rate. The effect of *in vitro* induced bulblet size on *ex vitro* survival rate has also been reported in Lachenalia. Transplanting of *in vitro* plantlets is more labor intensive than planting bulblets directly on soil. However, in order to successfully implement the use of *in vitro* generated bulblets rather than *in vitro* rooted plantlets of *Ornithogalum virens* for commercial production.

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# إكثار وتكوين أبصال الأيرس صنف البلوماجيك معمليا

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تعتبر المغذيات المعدنية والسكر ومنظمات النمو من العوامل المؤثرة علي نمو وتطور نباتات الايرس في مزارع الأنسجة النباتية . و يهدف هذا البحث إلي دراسة تأثير السيتوكينينات (البنزيل ادنينBA) والاكسينات ( نفتالين حامض الخليك NAA) وأيضا نوع البيئات المستخدمة ( موراشيج وسكوج MS وجامبورج B5 وبيئة النباتات الخشبية WPM ) علي النمو في المعمل أثناء مرحله التضاعف وأيضا تأثير مثبطات النمو( سيكوسيل CCC والبكلوبترازول PP333 و حامض الابسيسيك ABA ) والسكروز علي تكوين الأبصال. ولقد تم دراسة تأثير تفاعل البنزيل ادنين ونفثالين حامض الخليك المضاف إلي البيئة علي أبصال الأيرس في المعمل لتكوين الأفرع فوجد أن استخدام تركيز ١ أو ٢مليجرام /لتربنزيل ادنين و ٠.٢ مليجرام/لتر نفتالين حامض الخليك في بيئة الزراعة أعطي أفضل تكوين أفرع بقياس عدد الأفرع وعدد الأوراق وطول النبات والوزن الطازج. ولقد بينت الدراسة أن استخدام بيئة موراشيجي وسكوج أعطي أفضل نتيجة في تكوين الأفرع مقارنه باستخدام بيئة جابورج B5 وبيئة النباتات الخشبية WPM. ولقد ادي استخدام السيكوسيل CCC بتركيز ٢٥ مليمول في البيئة إلي الحصول علي اعلي عدد وأفضل نسبه تكوين للأبصال ، بينما استخدام تركيزات حامض الابسيسيك ABA المختلفة أعطت أفضل عدد أفرع وعدد أوراق بالمقارنة بباقي المعاملات. و تم دراسة تأثير التركيزات المختلفة من السكروز (٣٠و٦٠و٩٠جرام/لتر ) علي تكوين الأبصال فوجد إن استخدام بيئة مراشيج وسكوج MS المحتويه علي ٢٥ مليمول بكلوبترازول و٩٠ جرام سكروز ادت إلى زيادة في عدد الأبصال وقطرها ونسبه نجاح أقلمه النباتات في الصوبه(٩٠%). و في مرحله التجذير تم نقل النباتات إلي بيئات محتويه علي نوعين من الاكسينا ت (اندول حامض البيوترك وثفثالين حامض الخليك بتركيزات ٠.٠و١.٠و٢.٠و٣.٠ مليجرام/ لتر). وأظهرت التجارب إن استخدام بيئة الزراعة المحتوية علي ٢.٠مليجرام /لتر نفتالين حامض الخليك أعطي اكبر عدد وطول للجذور وأيضا أفضل نسبه نجاح في الاقلمه (٨٥%) في الصوبة وذلك باستخدام خليط من البيتموس والبيرليت بنسبه ١:٢في أقلمه النباتات .