The Effect of Plant Growth Regulators on In Vitro Production of Yellow Royal Dutch Iris Bulbs

Aya Al-ameri Mohammed Mehdi Muhsen Almasoody

Department Of Plant Production Techniques .Al-Musayyab Technical College
Al-Furat Al-Awsat Technical Universit Babylon, Iraq

Abstract

The study was conducted in the Plant Tissue Culture Laboratory, Department of Plant Production Technologies, College of Technology - Al-Musayyab, Al-Furat Al-Awsat Technical University, Babylon Governorate. The objective was to assess the impact of plant growth regulators (PGRs) on the regeneration of Yellow Royal Dutch Iris using in vitro tissue culture techniques. Three experiments were performed:

* .1 Shoot Regeneration*: Naphthalene Acetic Acid (NAA) at 0, 1, 1.5, and 2 mg/L was combined with Benzyl Adenine (BA) at 0, 1, 1.5, and 2 mg/L.

* .2 Root Induction*: NAA at 0, 0.3, 0.5, and 0.7 mg/L was combined with Indole-3-Butyric Acid (IBA) at 0, 0.5, 1, and 1.5 mg/L.

* .3 Bulb Formation*: NAA at 0, 0.3, 0.5, and 0.7 mg/L was combined with IBA at 0, 0.5, 1, and 1.5 mg/L. The experiments followed a Completely Randomized Design (CRD) with five replicates. Data were analyzed using the Least Significant Difference (LSD) test at a 0.05 probability level.

Introduction

The Iris genus, belonging to the Iridaceae family, encompasses a diverse array of visually striking flowering plants (Asgough et al., 2009). These plants, which can be either herbaceous or deciduous, are adapted to thrive in temperate climates globally (britannica, 2020). Iris species exhibit various growth habits, including bulbous, rhizomatous, and tuberous forms (Keppel, 1978). The Dutch Iris, a monocotyledonous plant, is characterized by its swollen bulb. While lacking a pronounced fragrance, Iris flowers are highly prized for their vibrant and varied coloration. The distinctive floral arrangement, coupled with the diversity in color patterns and leaf morphology, contributes to its popularity in the floriculture industry, particularly for premium cut flower production (Uzun et al., 2014). Beyond their ornamental value, various Iridaceae species have been utilized in both traditional and modern medicine for their therapeutic properties. Research has elucidated that these plants possess anti-inflammatory, antirespiratory, and anti-allergic properties, suggesting their potential in treating certain ailments (Rahman et al., 2003; Ayoub et al., 2018). Furthermore, they have applications in the production of cosmetic products, including soaps and perfumes (Wang et al., 1999; Jevremovic and Radojevic, 2002; Nacercilar and Deniz, 2014). Conventional propagation of Iris via seedlings presents considerable challenges due to the protracted seed maturation period (Simont, 1932). Consequently, traditional propagation relies heavily on vegetative reproduction using bulbs, a method that exhibits limitations in efficiency (Hassey, 1975; Jehan et al., 1994).

ISSN 2072-3857

Although each mother bulb can produce three to five new bulbs annually through vegetative propagation, this rate is still considered less efficient for large-scale production (Shibli and Ajlouni, 2000). Plant tissue culture, specifically micropropagation, has emerged as a transformative technology in plant propagation, enabling the rapid production of a large quantity of plants. This technique offers a means to propagate species that are recalcitrant to conventional propagation methods. Moreover, micropropagation facilitates year-round production, circumventing the limitations imposed by seasonal growth patterns. The ex vitro establishment of micropropagated plants typically involves a four-stage process: shoot initiation, multiplication, rooting, and acclimatization (Purohit, 2003). The composition of the culture medium, particularly the type and concentration of plant growth regulators (PGRs), is a critical determinant of success in plant micropropagation. Auxins and cytokinins are the most frequently employed PGRs in tissue culture, playing pivotal roles in in vitro bulb propagation (Chanteloube et al., 1995; Ramawat, 2004). This study aims to investigate the in vitro production of yellow Royal Dutch Iris bulbs, utilizing plant tissue culture techniques.

Materials and Methods

2.1 Plant Material: Yellow Royal Dutch Iris bulbs, sourced from the Netherlands, served as the initial explant material for this study. Upon arrival, the bulbs were thoroughly washed with distilled water and liquid soap to remove adhering debris. Subsequently, the bulbs were rinsed extensively under running tap water for 1 hour to eliminate residual soil particles. The cleaned bulbs were then transferred to a laminar airflow cabinet for surface sterilization.

2.2 Surface Sterilization : Surface sterilization of the bulbs was conducted within the laminar airflow cabinet using sodium hypochlorite (NaOCl) solutions. The bulbs were immersed in a 2% NaOCl solution for 15 minutes, followed by a 1-minute immersion in 70% ethanol. To remove any remaining sterilizing agents, the bulbs were rinsed five times with sterile distilled water. Sterilized bulbs were placed in sterile Petri dishes.

2.3 Culture Medium Preparation: The culture medium employed in this study was Murashige and Skoog (MS) medium (1962 formulation) obtained from Himedia. The medium was prepared by dissolving 4.9 g of MS powder and 7 g of agar (solidifying agent) in 1 liter of distilled water. Sucrose was added to the medium at a concentration of 3% (w/v). Plant growth regulators (PGRs), specifically Benzyl Adenine (BA), Naphthalene Acetic Acid (NAA), and Indole-3-Butyric Acid (IBA), were incorporated into the medium according to the concentrations specified in the experimental design. The pH of the medium was adjusted to 5.6 using either hydrochloric acid (HCl) or 0.1 M sodium hydroxide (NaOH). The medium was heated using a hot plate magnetic stirrer and microwaved to ensure homogeneity, dispensed into test tubes (10 mL per tube), and autoclaved at 121°C and 1.04 kg/cm² for 15 minutes. Following autoclaving, the medium was allowed to solidify at room temperature .

2.4Shoot Regeneration: Stem discs were excised from the sterilized bulbs and sectioned into four explants, which were then placed on the MS medium supplemented with varying concentrations of BA (0, 1, 1.5, and 2 mg/L) and NAA (0, 1, 1.5, and 2 mg/L) to induce shoot regeneration .

ISSN 2072-3857

2.5Root Induction: For root induction experiments, explants were cultured on MS medium supplemented with NAA (0, 0.3, 0.5, and 0.7 mg/L) and IBA (0, 0.5, 1, and 1.5 mg/L .(

2.6Bulb Production: To study bulb production, the MS medium was modified to contain varying concentrations of sucrose and IBA.

2.7 Culture Conditions: All cultures were incubated in a growth chamber maintained at 25 ± 2°C under a 16-hour photoperiod with a light intensity of 1000 lux.

2.8 Experimental Design and Data Analysis: The experiments were conducted using a completely randomized design (CRD) with five replicates per treatment. Data were analyzed using analysis of variance (ANOVA), and means were compared using the Least Significant .Difference (LSD) test at a significance level of $p \leq 0.05$.

.3Results and Discussion

3.1 Shoot Regeneration: The effect of Benzyladenine (BA) and Naphthalene Acetic Acid (NAA) on shoot regeneration of Iris was assessed, and the results are presented in Table 1. The highest number of shoots (4.95) was 0 observed at 2 mg/L BA in combination with 0 mg/L NAA. This result indicates a positive influence of BA on shoot proliferation. In contrast, the lowest number of shoots (1.10) was recorded in the absence of both BA and NAA (control). These findings align with previous studies demonstrating the stimulatory effect of cytokinins, such as BA, on axillary bud outgrowth and shoot development (Bruyn et al., 1992). BA's effectiveness in promoting shoot multiplication can be attributed to its role in overcoming apical dominance and stimulating the release of lateral buds. However, increasing the concentration of NAA tended to suppress shoot formation. This inhibitory effect of higher NAA concentrations may be due to hormonal imbalances caused by auxin accumulation in the explants, potentially leading to the inhibition of lateral bud development due to the promotion of apical dominance (Mohsen, 2009). High auxin levels can disrupt the vascular connections between the axillary buds and the stem, limiting nutrient translocation and hindering bud growth (Kim et al., 1991.(

Figure (1) Shoot Regeneration

Table (1) Effect BA & NAA on Shoot regeneration

NAA mg/l					
BA mg/l	**0**	**1**	**1.5**	**2**	**Mean BA**
0	**0.00**	**1.40**	**1.40**	**1.60**	**1.10**
1	**6.20**	**2.40**	**3.20**	**2.40**	**3.55**
1.5	**6.80**	**2.20**	**3.60**	**3.20**	**3.95**
2	**10.00**	**3.80**	**3.00**	**3.00**	**4.95**
Mean NAA	**5.75**	**2.45**	**2.80**	**2.55**	
L.S.D 5 % BA= 1.065 NAA= 1.065 BA* NAA=2.131					

3.2

Root Induction: The influence of NAA and IBA on root induction is shown in Table 2. The highest number of roots (4.25) was observed at 0.7 mg/L NAA, while the control (0 mg/L NAA and 0 mg/L IBA) produced significantly fewer roots (0.65). Similarly, 1.5 mg/L IBA resulted in a higher number of roots (4.60) compared to the control (1.45). These results confirm the well-established role of auxins, including NAA and IBA, in adventitious root formation (Atteya et al., 2018; Daniel et al., 2019). Auxins promote root initiation by stimulating cell division and elongation in the root primordia. These findings are consistent with previous reports on auxin-induced rooting in other plant species (Pandey et al., 2016). The interaction between NAA and IBA was also significant. The combination of 0.7 mg/L NAA and 1.5 mg/L IBA yielded the highest number of roots (7.40), suggesting a synergistic effect of these two auxins on root development.

ISSN 2072-3857

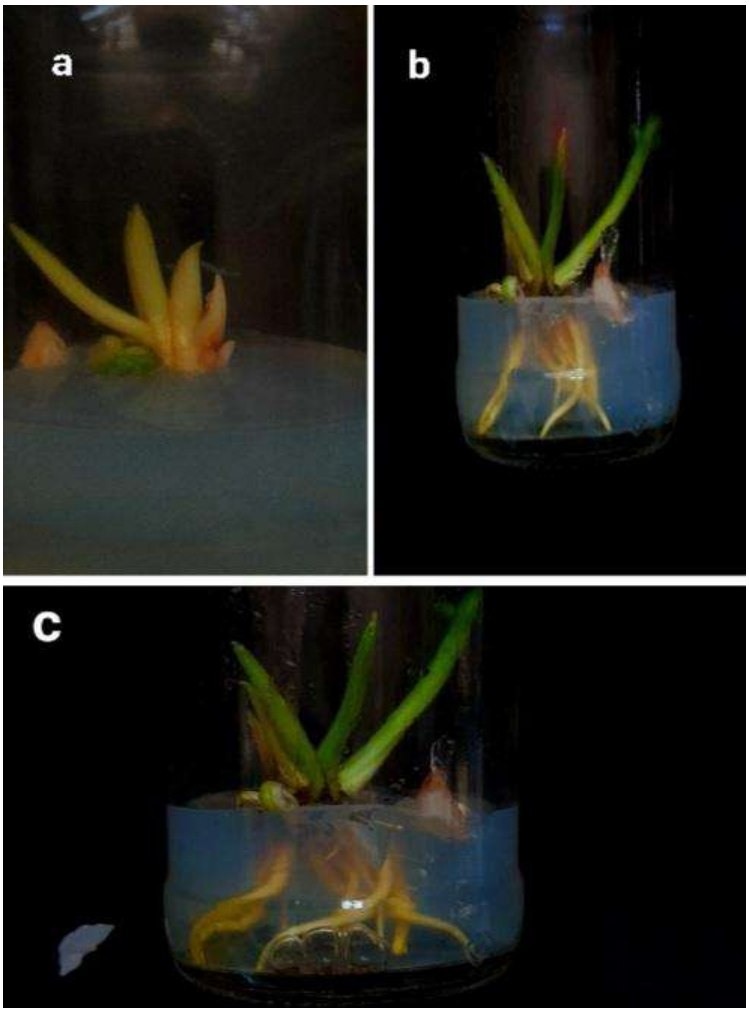

Figure (2) Root Induction

Table (2) Effect NAA & IBA on Root induction

IBA mg/l					
NAA mg/l	**0**	**0.5**	**1**	**1.5**	**Mean NAA**
0	**0.00**	**0.40**	**0.80**	**1.40**	**0.65**
0.3	**1.20**	**3.00**	**3.20**	**3.60**	**2.75**
0.5	**2.00**	**4.00**	**4.40**	**6.00**	**4.10**
0.7	**2.60**	**3.60**	**3.40**	**7.40**	**4.25**
Mean IBA	**1.45**	**2.75**	**2.95**	**4.60**	
NAA=0.843 IBA 0.843 L.S.D 5% **NAA*IBA = 1.686**					

3.3

Bulb Production: The influence of sucrose concentration and IBA on bulb production is presented in Table 3. The highest number of bulbs (1.650) was observed at 9% sucrose, indicating that higher sucrose concentrations promote bulb formation. Similarly, 1 mg/L IBA resulted in a higher rate of bulb formation (1.950). The interaction between sucrose and

ISSN 2072-3857

IBA was also significant, with the combination of 6% sucrose and 1.5 mg/L IBA yielding the highest bulb formation rate (3.600). Sucrose plays a crucial role in tissue culture as a carbon source and in osmoregulation. Elevated sucrose concentrations can induce a physiological state that leads to the accumulation of metabolites, which can manifest as swelling and bulb formation (Ziv, 1990). The optimal sucrose concentration for bulb formation varies among plant species, as reported in previous studies (Ali, 2005 .(

Figure (3) Bulb Production

Table (3) Effect of sucrose & IBA on Production of bulbs

IBA mg/l					
sucrose %	**0**	**0.5**	**1**	**1.5**	**Meansucrose**
3	**0.000**	**0.400**	**0.800**	**1.000**	**0.500**
4.5	**0.200**	**1.000**	**0.800**	**1.400**	**0.850**
6	**1.000**	**1.400**	**1.800**	**3.600**	**1.150**
9	**0.800**	**0.600**	**1.200**	**0.600**	**1.650**
Mean IBA	**0.550**	**0.850**	**1.950**	**0.800**	
L.S.D 5%		**sucrose= 0.4868**		**IBA=0.4868**	
sucrose* IBA=0.9736					

ISSN 2072-3857

References

.1 Ali, W.A.S.A. 2005. Studies on micropropagation of nightshade bulbs. Master's thesis. Faculty of Agriculture, Ain Shams University, Egypt.

.2 Asgough, G.D.J.E.; Erwin, J.E.; Staden, J.V. 2009. Micropropagation of Iridaceae: A review. Plant Cell Tissue Organ Culture 97:1-19.

.3 Atteya, A.K.G.S.K.; Al-Taweel, E.A.E.; Genaidy, H.A.Z. 2018. Effect of Gibberellic Acid and zinc sulphate on Vegetative, Flowering, Seed yield and Chemical Consistent Of Jojoba Plant (Simmondsia Chinensis). India Journal of Agricultural Research 52(5):542-547.

.4 Ayoub, I.M.; Korinek, M.; Hwang, T.L.; Chen, B.H.; Chang, F.R.; ElShazly, M.; Singab, A.N.B. 2018. Probing the antiallergic and antiinflammatory Activity of Biflavonoids & Dihydroflavonols From Dietes Bicolor. Journal of Natural Products 81:243-253 .

.5Bruyn, D.M.I.D.; Ferreira, M.M.; Slabbert, M.M.; Pretorius, J. 1992. In vitro Propagation of Amaryllis Belladonna. Plant Cell Tissue and Organ Culture 31:184-189 .

.6The editors of encyclopaedia. 2020 Sep 10. Iridaceae. Encyclopaedia Britannica.

.7Chanteloube, F.; Courduroux, J.; Tort, M.; Le Nard, M. 1995. Micropropagation of Tulipa Gesneriana L. Regeneration of bulblets on growing floral stem segments cultured in vitro. Acta Botanica Gallica 142(4):301-307.

.8 Danial, G.H.D.A.; Ibrahim, A.N.Y.; Yousef, S.B.E. 2019. Rapid Protocol Of Aloe Vera In Vitro Propagation. Iraqi Journal of Agricultural Sciences 50(5):1377-1382.

.9 Hussey, G. 1975. Totipotency in Tissue Explants and Callus of Some Members of the Liliaceae, Iridaceae And Amaryllidaceae. Journal of Experimental Botany 26:253-262 .

.10Jehan, H.; Courtois, D.; Ehret, C.; Lerch, K.; Petiard, V. 1994. Plant regeneration of Iris Pallida Lam. and Iris Germanica L. via somatic embryogenesis from leaves, apices and young flowers. Plant Cell Reports 13:671-675 .

.11Jevremovic, S.; Radojevic, L.J. 2002. Plant regeneration from suspension cultures of Iris Pumila L. Acta Horticulturae 572:59-65.

.12 Keppel, K. 1978. The Xiphiums. The World of Irises. 277-281 .

.13Kim, K.W.; Kang, M.S.; Goo, D.H. 1991. The external and histological characteristics of organogenesis from Gladiolus callus. Korean Society for Horticultural Science 32:124-129.

.14 Mohsen, M.M. 2009. Propagation of Gladiolus sp. using tissue culture technique. Master's thesis. Al-Musayyab Technical College, Al-Furat Al-Awsat Technical University, Republic of Iraq.

.15 Nasircilar, A.G.; Deniz, I.G. 2014. An alternative plant Propagation And conservation process for Iris pampyhlica an endemic and endangered geophyte. Fifth International Scientific Agricultural Symposium Agrosym .

.16Pandey, R.K.; Singh, A.K.; Sharma, M. [Year not found]. Propagation of Lilium. Biological Forum-International Journal 1(2):26-28.

.17 Purohit, S.S. 2003. Plant tissue culture. Shyam Printing Press: Jodhpur. p. 153-175 .

.18Rahman, A-U.; Nasim, S.; Baig, I.; Jalil, S.; Orhan, I.; Sener, B.; Choudhary, M.I. 2003. Anti-inflammatory Isoflavonoids From the Rhizomes Of Iris Germanica. Journal of Ethnopharmacology 86:177- 180 .

.19Ramawat, K.G. 2004. Plant Biotechnology. India. p. 1-265.

ISSN 2072-3857

.20 Shibli, R.A.; Ajlouni, M.M. 2000. Somatic embryogenesis in the endemic black Iris. Plant Cell Tissue Organ Culture 61:15-21.

.21 Simonet, M. 1932. Plant REgeneration Of Als Pallida Lam. And ins germanica L. Via somatic embryogenesis from leaves, apices and Young flowers. Bulletin Biologique de la France et de la Belgique 105:255-444 .

.22Uzun, S.; Ilbaş, I.; Ipek, A.; Arslan, N.; Barpete, S. 2014. Efficient in Vitro plant regeneration from immature embryos of endemic Iris sari and I. schachtii. Turkish Journal of Agriculture and Forestry 38:348-353 .

.23Wang, Y.; Jeknic, Z.; Ernst, R.C.; Chen, T.H. 1999. Improved plant regeneration from suspension-cultured cells of Iris germanica l'skating Party. HortScience 34:1271-1276.

.24 Ziv, M.; Lilien-Kipnis, H. 1990. Gladiolus. In: Evans, D.A.; Sharp, W.R.; Ammirato, P.V.; Yamada, Y.Y., editors. Handbook of Plant Cell Culture 5:461-478.

ISSN 2072-3857