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Influence of Auxins and different growth media strength on rooting of *Petunia hybrida* L. *In vitro* Propagation.

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ABSTRACT

This experiment was carried out in the Plant Cells and Tissue Culture Laboratory of Horticulture and Landscaping Design Department/College of Agriculture/ University of Kirkuk-IRAQ, to study the effect of auxin's in different concentrations and MS media (full and ½) salt strength on rooting response of *Petunia hybrida*. The regenerated shoots explants from the multiplication stage were transferred to the rooting stage cultured on MS media (full and ½) salts strength supplemented with different concentrations of IBA at (0.0, 0.25, 0.50, 0.75, and 1.0) mg L^{-1}, and NAA at (0.0, 0.25, 0.50, 0.75, and 1.0) mg L^{-1}. After 6 weeks of transforming, data showed that the response percentage was (100%) for MS media (full and ½) salt strength supplemented with different concentrations of IBA and NAA beside control treatment. The best number of roots (29.70 root.part^{-1}) and the length of the longest root (7.44 cm) were recorded by MS ½ strength supplemented with IBA 1.0 mg L^{-1} compared with other concentrations, and it was the highest results compared with MS full strength supplemented with different concentration of IBA. Moreover, results obtained that MS ½ strength supplemented with NAA 1.0 mg L^{-1} recorded the best number of roots (18.70 root.part^{-1}) and the length of the longest root (2.97 cm) compared with the other concentrations. At the same time, it was the best result compared with MS full strength supplemented with different concentrations of NAA.

***Keywords*:** Auxin, Petunia hybrida, MS، In Vitro, IBA, NAA.

INTRODUCTION

Ornamental plants are considered an economic product used in the local markets or for export to foreign markets. It occupies an important position in home decorations. It adds a beautiful painting [1]. Petunia is grown on a very large scale worldwide because of their ornamental value due to the variety of flower colours and its long-lasting flowers [2]. *Petunia hybrida*, which originated from South America, it is variable in shape, and color and very popular in the world, it is cultivated as a potting and bedding flower to decorate the landscape. It was considered one of the most common perennial herbaceous ornamental plants in the world [3]. In the United States *Petunia hybrida* flowers occupy at the forefront of herbaceous annual plants in terms of aesthetic value [4]. Among the developed modern plants, Petunia hybrida is a classic variety due to crossing between two other species, *Petunia axillaris* × *Petunia integrifolia* [5]. Petunia is a genus of 35 species of the Solanaceae family, with a height of 35 cm for the shoot, their leaves are oval and round, with a green colour, and have very big flowers. It is reproduced by seeds and stem cuttings, that is widely cultivated in temperate and sub-tropical regions [6]. Economically important plants are propagated by tissue culture [7]. Tissue culture techniques are used for micropropagation and production of disease-free plants [8] Plant tissue culture has come out as a promising method, it forms a base for plant biotechnology with the increasing demand of farmers for high-quality agricultural materials to increase production. The tissue culture method is processed by explanting sterile plant parts on a media (MS) under controlled conditions. Plant tissue culture is a technique using different parts of growing plant cells, organs, tissues, seeds, and other plant parts in an aseptic and controlled environment inside a laboratory [9] Different concentrations of growth regulators are used to produce plant parts and control growth inside the media. Auxins are considered one of the basic plant growth regulators are responsible for one or more morphological processes in plants like the formation of the shoot and root system in plants [10], [11]. The rooting response of regenerated from in vitro shoots depends on auxin type and concentration inside the MS media [12]. Indole butyric acid (IBA) and Naphthalene acetic acid (NAA) are the most common auxins used for rooting [13], [14]. This study aimed to find suitable growth regulators and the best MS strength for root initiation from in vitro multiplication of *petunia hybrida* shoot explants.

Material and Methods:

distilled water consecutively for 5 minutes. For multiplication, two plant growth regulators were used on the MS media, Benzyl adenine at concentrations (0.0, 0.5, 1.0, 1.5, and 2.0) mg L^{-1} and Kin at concentrations (0.0, 2.0, 4.0, 6.0, and 8.0) mg L^{-1} for multiplication stages. After (8) a week, shoots cuttings transferred from multiplication stage to root formation in MS basal media at (full and half) strength supplemented with different concentrations of IBA at concentrations (0.0, 0.25, 0.50, 0.75, and 1.0) mg L^{-1} and NAA at concentration (0.0, 0.25, 0.50, 0.75, and 1.0) mg L^{-1}, added to 0.7% g/l agar + 3% (w/v) sucrose inside glass water contains 600 ml distilled on a rotating heater. After boiling and dissolving, the agar was supplemented with MS basal media at (full 4.43 g) and (half 2.21 g) strength to the

homogeneity solution, the volume was was used for the experiment, averages were compared to the T-test by using polynomial Duncen's multiple range test under the level of 5% probability. With ten replications for each treatment, each replicate consisted of one plant part [15]. All analysis was done using SAS statistical software [16].

Results and Discussions

Effect of MS media with (full and ½) strength of salts supplemented with different concentrations of IBA on rooting of *petunia hybrida* after 6 weeks:

Data obtained in Table (1) cleared that, after 6 weeks of culturing *petunia hybrida* explants from the multiplication stage on MS media with (full and ½) strength of salts supplemented with different concentrations of IBA, the response percentage was 100% for all concentration of IBA and both strength (full and ½) MS media for *petunia hybrida*. Moreover, the statistical analysis after 6 weeks showed that there was a significant increase in several roots with increasing IBA concentration, MS media with full strength supplemented with 1.0 mg L^{-1} IBA handed the highest rate for the number of roots reached (17.90 root.part^{-1}) compared other concentration, there was a significant difference with the control treatment. Furthermore, data revealed that the best rate of length of the longest root (4.13 cm) was obtained at 1.0 mg L^{-1} IBA compared with the other concentrations. Results in Table (1) revealed that after 6 weeks of adding IBA at a concentration 1.0 mg L^{-1} to the MS media with ½ strength of salts increased the number of roots achieved (29.70 root.part^{-1}) compared with the control treatment which caused the lowest rate 4.8 root.part^{-1}. Moreover, data showed that the length of the longest root characterized at concentration 1.0 mg L^{-1} increased the length of the longest root recorded (7.44 cm) compared with other concentrations

Table (1): Effect of MS media with (full and ½) strength supplemented with different concentrations of IBA on rooting of *petunia hybrida* after 6 weeks.

IBA mg L^{-1}	MS with full strength			MS with ½ strength		
	Response %	NO. of roots Root/plant	Length of the longest root (cm)	Response %	NO. of roots Root/plant	Length of the longest root (cm)
0.0	100	5.20 D	2.00 b	100	4.8 c	1.75 C
0.25	100	8.80 C	1.39 b	100	13.90 b	2.02 Bc
0.50	100	11.40 Bc	1.48 b	100	20.50 b	3.06 B
0.75	100	12.80 B	1.65 b	100	27.70 a	3.10 B
1.0	100	17.90 A	4.13 a	100	29.70 a	7.44 A

The averages with similar letters for each factor separately and their interactions did not differ significantly according to Duncan's polynomial test at the 5% probability level

Effect of MS media with (full and ½) strength of salts supplemented with different concentrations of NAA on rooting of *petunia hybrida* after 6 weeks:

When shoots were cultured on MS basal media, data revealed that, after 6 weeks of culturing *petunia hybrida* explants from the multiplication stage on MS media with (full and ½) strength of salts supplemented with different concentrations of NAA, the response percentage was 100% for all concentration of NAA and both strength (full and ½) MS media for *petunia hybrida*. Moreover, the statistical analysis after 6 weeks showed that there was a significant increase in several roots with increasing of NAA concentration, MS media with full strength supplemented with 1.0 mg L^{-1} NAA handed the highest rate for the number of roots reached (12.00 root.part^{-1}) compared other concentration, there was a significant difference with the control treatment. Furthermore, data revealed that the best rate of length of the longest root (2.76 cm) was obtained at 1.0 mg L^{-1} NAA compared with the other concentrations.

Results in Table (2) revealed that after 6 weeks of adding NAA at concentration 1.0 mg L^{-1} to the MS media with ½ strength of salts increased the number of roots achieved 18.70 root.part^{-1} compared with the control treatment which caused the lowest rate of 4.8 root.part^{-1}. Moreover, data showed that the length of the longest root characterized at concentration 1.0 mg L^{-1} increased the length of the longest root recorded (2.97 cm) compared with other concentrations.

Table (2): Effect of MS media with (full and ½) strength supplemented with different concentrations of NAA on rooting of *petunia hybrida* after 6 weeks.

NAA mg L^{-1}	MS with full strength			MS with ½ strength		
	Response %	NO. of roots Root/plant	Length of the longest root (cm)	Response %	NO. of roots Root/plant	Length of the longest root (cm)
0.0	100	5.20 C	2.00 b	100	4.8 c	1.75 bc
0.25	100	5.80 C	1.28 c	100	10.30 b	1.32 c
0.50	100	8.20 B	1.76 bc	100	12.00 b	1.80 bc
0.75	100	9.70 B	2.21 ab	100	17.60 a	2.28 b
1.0	100	12.00 A	2.76 a	100	18.70 a	2.97 a

The averages with similar letters for each factor separately and their interactions did not differ significantly according to Duncan's polynomial test at the 5% probability level.

Discussion

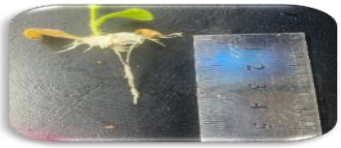
Data obtained in Table (1) and (2) cleared the effect of auxin (IBA and NAA) and MS media at (full and ½) salt strength on rooting response of in vitro regenerated shoots which is a very important stage in tissue culture. the growth regulator auxin is a natural hormone found in the growing apical of plants, and it plays an important role in cell division, elongation, and differentiation [17]. Auxin has a major role in adventitious root formation through its physiological effect in losing the differentiation of specialized parenchymal cells and returning them to the meristematic state through the process of (dedifferentiation) which in turn form roots initially through divisions which grow and develop to the root primordium, which grows out of the stem tissue, forming the adventitious [18]. The effect of auxin on the elasticity and plasticity of the cells causes elongation in cells and increases their size due to the entrance of water into them. Auxins dissolve organic calcium pectate and its mineral ions, which cause the hardening of cell walls. They also disintegrate and shatter some organic materials, such as pectin, cellulose, and hemicellulose, which are responsible for cell wall adhesion and cohesion as a result of the activation of hydrolytic enzymes such as cellulase and pectinase [19]. The role of low concentrations of auxins and MS media with half strength can be explained as, auxins help cells divide and grow and stimulate the formation of Ribonucleic acid (RNA). The mRNA provides energy through its activity which is related to the process of oxidation of nutrients and the formation of enzymes related to growth, including respiratory enzymes that produce the compound ATP in large quantities that are exploited by tissues for division and growth purpose. These results are in agreement with [20], [21], [22], [23], and [24] Moreover, the superiority of IBA in root formation due to that IBA is considered one of the auxins that play a fundamental and important role in activating or stimulating the process of cell division and elongation, it also plays a role in stimulating the formation of roots in the cutting areas, the use of growth regulators and adding them to the media works to increase the number of roots and their lengths to reach the optimal concentration, and increasing the concentration leads to adverse effects [25]. In addition, the role of auxins in increasing cellular permeability, ion exchange, and enzymatic activity, they also increase the transfer of nutrients that lead to inducing root formation, which works to increase the rate of root formation on the planted parts, due to the important and direct role it plays in the rooting stage [26]. The effect of MS media at (half) salt strength on the rooting response of in vitro regenerated shoots, that even when auxins are present in the culture media, high salt concentrations may prevent root growth [27]. During the rooting phase of most plant species, it is important to reduce the concentration of macro and micronutrients to half of their typical concentrations [28]. This could be due percentage of nitrogen in the MS media has decreased, the use of low concentrations in the nutritional media has been mentioned by many researchers, especially in the MS medium half salts strength, as it contains high concentrations of ammonia and nitrogen ions, which help stimulate the tips of plant branches resulting from tissue culture to form roots. [29]. These results are in agreement with [30] and [31]

Cont

IBA 0.25 Full

IBA 0.50 Full

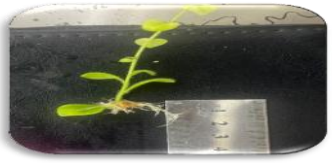
IBA 0.75 Full



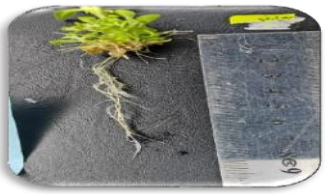
IBA 1.0 Full

Cont.

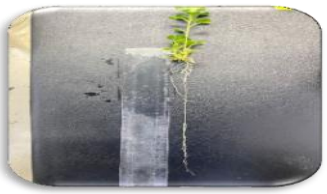
IBA 0.25 Half



IBA 0.50 Half



IBA 0.75 Half



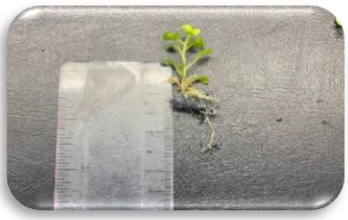
IBA 1.0 Half

CTRL

NAA 0.25 Full

NAA 0.50 Full

NAA 0.75 Full



NAA 1.0 Full

CTRL

NAA 0.25

NAA 0.50 Half

NAA 0.75 Half

NAA 1.0 Half

Conclusion

Results showed that IBA and NAA (PGRs) at 1.0 mg L^{-1} significantly increased the number of roots and their length compared with other concentrations under study for both media (full and half) strengths.

Recommendation:

The researcher recommends using IBA and NAA with low concentrations on other ornamental plants as it promotes and elongation plants roots.

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تأثير الأوكسين وقوة الأوساط الغذائية في تجذير البتونيا الهجين *Petunia hybrida* L. بالإكثار خارج الجسم الحي.

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الخلاصة

أُجريت هذه التجربة في مختبر زراعة الخلايا والأنسجة النباتية التابع لقسم البستنة وهندسة الحدائق / كلية الزراعة / جامعة كركوك - العراق، لدراسة تأثير الأوكسين ***IBA*** بتركيز (0.0، 0.25، 0.50، 0.75، و 1.0) ملغم لتر$^{-1}$، و ***NAA*** بتركيز (0.0، 0.25، 0.50، 0.75، و1.0) ملغم لتر$^{-1}$ وقوة أملاح الوسط (الكاملة والنصف) على تجذير نباتات البتونيا الهجينة ***Petunia hybrida L.*** بالإكثار خارج الجسم الحي. أظهرت النتائج بعد 6 أسابيع من نقل الأجزاء الخضرية من مرحلة التضاعف إلى مرحلة التجذير أن نسبة الاستجابة كانت (100%) للوسط ***MS*** بكلا القوتين المضاف إليه التراكيز المختلفة من الـ ***IBA*** و ***NAA*** بالإضافة إلى معاملة السيطرة. وتفوق الوسط ***MS ½*** قوة المزود بـ ***1.0*** ملغم لتر$^{-1}$ ***IBA*** في صفة عدد الجذور (29.70 جذر/جزء نباتي) وطول أطول جذر (7.44 سم)، وحققت أعلى النتائج مقارنة مع الوسط ***MS*** بقوة أملاح كاملة. في حين أن الوسط ***MS*** بـ ½ قوة أملاح والمزود بـ ***1.0*** ملغم لتر$^{-1}$ ***NAA*** أعطت أفضل عدد للجذور (18.70 جذر/جزء نباتي) وطول أطول جذر (2.97 سم) مقارنة بالتراكيز الأخرى، وأعطت أفضل نتيجة مقارنة مع الوسط ***MS*** بقوة أملاح كاملة.

الكلمات المفتاحية: أوكسين، البتونيا، ***MS***، الأكثار خارج الجسم الحي، إندول حامض الخليك ، نفثالين حامض الخليك.