Effect of nutrient media enhanced with plant-growth regulators on indirect somatic embryogenesis induction for the tissue culture of *Digitalis purpurea*

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### ABSTRACT

The aim of this study was to establish effective protocols in indirect somatic embryogenesis induction for the tissue culture of *Digitalis purpurea* in several nutrient mediums enhanced with plant-growth regulators and in the presence of light and dark factors. The results show the superiority of the Volosovich (5C01) medium in terms of the days required for callus formation compared to the Murashige and Skoog (MS), Linsmaier and Skoog (LS), and Gamborg (B5) media in dark and light conditions. According to the callus induction index, the leaf explants were superior to the stem and root explants. Moreover, leaf explants had the highest number of indirect somatic embryos in the MS medium, with 12.50 and 14.25 in the dark and light, respectively. On the other hand, the 5C01 medium was superior for the callus induction and formation index. The results show a 95% callus induction rate at a concentration of 2 mg/L of NAA+1 mg/L of Kin in the dark and light treatments compared to all the studied treatments in the MS, LS, and B5 mediums. At a concentration of 0.25 mg/L 2,4-D+2 mg/L BA, the MS medium was better for the induction and formation index of indirect somatic embryos of the studied Callus induction from leaf explants by 52.75% in the dark treatments and by 90% in the light treatments.

### 1. INTRODUCTION

*Digitalis purpurea* is one of the most important plants of high medicinal value. It is a biennial herbaceous plant belonging to the Plantaginaceae family [1]. The leaves of *D. purpurea* were included in the London Pharmacopoeia in 1650 [2]. The first medical, scientific experiments for its use as a reliable treatment for heart patients date back to 1785, conducted by Doctor William Withering [3,4]. The medical importance of *D. purpurea* is that it contains cardiac glucosides, such as digoxin, used in treating congestive heart failure.

Consequently, in 1990, the FDA approved its use as a treatment for heart failure and atrial fibrillation. The effectiveness of digoxin is due to its effect on the sodium-potassium pump through its effect on the vagus nerve [5]. Several studies on the efficacy of digoxin in the treatment of breast and prostate cancer have recently been published [6,7]. In addition, a number of scientific reports stated that the antiviral effects of digoxin and some other cardiac glucosides, such as Ouabain, block Cov-2 transcription, or replication, which lead to the inhibition of the virus post-viral life cycle. Hence, recent research results indicate that digoxin and Ouabain may be alternative and effective treatments as antidotes to COVID-19, with potential additional therapeutic effects for patients with cardiovascular disease [8]. However, the difficulties in synthetic chemistry regarding the complexities of the skeletal structure of cardiac glucosides and the impossibility of their chemical synthesis make plants the only sources from which to obtain these cardiac glucosides [9]. Such traditional cultivation methods are no longer feasible in light of the challenges faced with traditional methods of propagation as well as the seasonal and environmental conditions. Verma et al. [9] reviewed the futility of the natural propagation of digitalis seeds, as this is an ineffective method for producing an adequate stock of seeds and is associated with a low germination rate and other risks. Probert et al. [10] reviewed the difficulties that affect the quality of seeds, such as processing methods and storage conditions. Consequently, researchers attempted to produce digitalis containing high glucosides through traditional cultivation. Still, the offspring were not stable, and repairing the traits required long-term programs that were cumbersome and expensive [10].

Therefore, biotechnological research in recent decades has focused on techniques of the tissue culture of digitalis species as an approach that goes beyond seasonal restrictions, overcomes the difficulties associated with traditional agriculture, and achieves sufficiency for the increasing human demand.
Somatic embryo propagation is one of the most effective techniques for the micropropagation of medicinal plants, a valuable technology for the production of clonal plants, and a promising tool for exploring diversity and highlighting many new and valuable properties [11]. Somatic embryogenesis induction occurs directly from explants or indirectly through the callus stage [12-14].

The callus-differentiation stage is the key to establishing the in vitro indirect regeneration system [15]. The indirect somatic embryo induction process occurs through an organized series of distinct embryonic stages, such as spherical, cardiac, and torpedo [5]. The success of indirect somatic embryo induction depends on factors such as the plant type, cultivation conditions, explant types, nutrient medium, plant-growth regulators, materials and other additives, and heat and light factors [16,17].

2. MATERIALS AND METHODS

2.1. Research Location

The research was carried out at the Biotechnology Laboratory of Medicinal Plants, the National Commission for Biotechnology, Damascus, Syria, and at the Tissue Culture Laboratories, Department of Plant Biology, Faculty of Science, Damascus University, Syria, during the period of 2018–2021.

2.2. Plant Material

The studied explants (leaves, stems, and roots) were obtained from in vitro plantlets of the studied species, *D. purpurea*, grown in glass (T = 25 ± 1°C; light = 2000 Lux 16/8; E = 30 days; H = 70) at the Laboratory of Medicinal Plant Biotechnology at the National Commission for Biotechnology, Syria.

2.3. Preparation of Nutrient Media

The nutrient media were prepared—MS [18], B5 [19], LS [20], and 5C01 [21]—and sterilized in an autoclave (T = 121°C; P = 1.2 Bar). These nutrient media were supplemented with a series of plant-growth regulators, auxins (2,4D, NAA, and IAA), and cytokinin (Kin, BAP, and BA) in graded concentrations (0.25, 0.5, 1, and 2 mg/L) and single and compound cases. Explants (leaves, stems, and roots) were planted on the studied media at a rate of 20 explants for each treatment (concentration) and four replications in each of the studied media (pH = 5.8; agar = 8 g/l; sucrose = 3%). Cultivation was carried out under sterile conditions in a JSCR-5C01 laminar device. Then, the cultivated explants were incubated in an autoclave (T = 121°C; P = 1.2 Bar). These data were analyzed using the Mstat-C statistical analysis program to calculate the values of the least significant difference at a 0.01% level of significance and the values of the coefficient of variance (CV%).

3. RESULTS AND DISCUSSION

3.1. Days Required for Callus Formation

The days required for the callus formation response varied according to the medium type, growth regulators, explant type, and explant level within the same species. Table 1 indicates the efficiency of the 5C01 and MS mediums in terms of the day’s index required for callus formation in the dark conditions compared to the B5 and LS mediums. Furthermore, the superiority of the leaf explants in all the studied nutrient mediums compared to the stem and root explants.

These results are agreed with the results of Besher [22] on the superiority of the 5C01 medium in inducing a callus response from *Hyoscyamus aureus*. Furthermore, the results agree with the findings of Zang et al. [15] regarding the superiority of the MS medium in inducing a callus response from the leaves of *Digitalis hamiltoni*.

Table 1 shows the superiority of the 5C01 medium, followed by the MS medium, in terms of the callus formation index in the light compared to the B5 and LS media, as well as the superiority of the leaf explants compared to the stem and root explants in the callus formation index of the studied nutrient media. Moreover, it can be noticed that light contributed to inducing the formation of Calli in the MS, B5, and LS media compared to their formation in the dark. In contrast, the light slowed down the induction of the formation of calluses in the 5C01 medium. This result may be due to the internal interactions between the medium components under the influence of light. Therefore, the effect of light on the total concentration of growth regulators (internal and external) was reflected in the induction response and its temporal timing. These findings agree with the results of Chen et al. [17] on the

| Treatment | Required days for starting callus formation. | Light 16/8 | Leaf | Stems | Roots | Mean | Leaf | Stems | Roots | Mean |
|-----------|------------------------------------------------|-----------|------|-------|-------|------|------|-------|-------|------|
|            | Leaves | Stems | Roots | Mean | Leaves | Stems | Roots | Mean |
| MS (DPM1)  | 13     | 16    | 14    | 14.33<sup>a</sup> | 12     | 15    | 14    | 13.66<sup>c</sup> |
| B5 (DPM2)  | 21     | 24    | 26    | 23.66<sup>a</sup> | 19     | 22    | 24    | 21.66<sup>c</sup> |
| LS (DPM3)  | 14     | 18    | 18    | 16.66<sup>c</sup> | 14     | 17    | 18    | 16.33<sup>c</sup> |
| 5C01 (DPM4) | 9      | 9     | 12    | 10<sup>c</sup>    | 12     | 13    | 14    | 13<sup>c</sup>    |
| Mean       | 14.25<sup>a</sup> | 16.75<sup>a</sup> | 17.5<sup>c</sup> | 14.25<sup>a</sup> | 16.75<sup>a</sup> | 18<sup>c</sup> |

Means in the table are followed by different letters; the letters represent the order of the mean value which starts from the best value (a) and decline, respectively, according to the letter order.
effect of light on the induction and formation of somatic embryos in
Howorthia and the effect of light on the activity of growth regulators
and the modification of internal hormone levels.

3.2. Indirect Somatic Embryogenesis Induction

Table 2 reveals the superiority of the 5C01 and MS media regarding
the number of days needed to form indirect somatic embryos in dark
conditions compared to the LS and B5 media, in addition to the
superiority of the leaf explants as a source of indirect somatic embryo
induction compared to the rest of the other studied explants in the dark
conditions. These results are in agreement with the results of Verma
et al. [23] that the calluses induced from leaf explants of Crocus species
on the MS medium formed somatic embryos earlier than in the
other explants and that the MS medium was the best medium for the
development of somatic embryos compared to the B5 and LS media.

The results presented in Table 2 show the superiority of the MS medium,
followed by the LS and B5 media, compared to the 5C01 medium
regarding the number of days needed for somatic embryo formation in
the light cycle 16, as well as the preference for the leaf explants compared
to the rest of the studied explants. The results of this study agree with
the results of [24], indicating that light is one of the most critical factors
controlling plant growth and development, as well as the results of
Farhadi et al. [25], indicating that light is one of the most significant
factors affecting the regeneration and micropropagation systems in a
number of crops, where the interactions among light, endogenous auxins,
and cytokinin directly or indirectly affect the regeneration systems in
plants [17].

3.3. Somatic Embryo Formation Percentages

The results presented in Table 3 show the superiority of the MS
medium regarding the indirect somatic embryo formation percentages
in the dark compared to the rest of the studied media, with higher
percentages of calluses induced from the leaf explants compared to the
other studied explants in the dark.

On the other hand, the MS and LS media were superior regarding the
rate of somatic embryo formation compared to the studied B5 and 5C01
medium in the presence of the light factor. The leaf explants were superior
to the other studied explants in the light condition. This may be due to
the leaves being the newest part of the plant, which is consistent with the
results of Verma et al. [23] regarding Crocus species, as the newer parts
were the most responsive to somatic embryogenesis and plant renewal.
The results also agree with the results of Krishnan et al. [26], indicating
that the callus induction average varied according to the type of explant,
while the differences in the response between the nutrient media were
due to the difference in nitrogen source and quantity, which is consistent
with the results of Mandal and Laxminarayane [11].

In comparing the light and dark factors, the results presented in Table 3
show significant differences that confirm the preference for light
conditions in inducing somatic embryos. This may be due to the effect
of light on the medium components, specifically on the growth regulators,
and stimulating internal hormones. In addition, light works to form
the embryonic callus, which is preferred for inducing indirect somatic
embryos. These results agree with the results of [27,28] on the importance
of light in inducing the development of indirect somatic embryos from
Oncidium callus cultures. Moreover, our results agree with those of
Verma et al. [23] about the superiority of the response rate of leaf explants
on the MS medium for Crocus oliveri compared to other studied media.

The results of this study are in consonance with the earlier report of
Gural et al. [29] on Digitalis davisciana Heywood about the presence of
significant differences in bud renewal depending on the explant type, plant
type, hormonal combination, and components of the basal medium. The
results also agree with those of other studies showing that calluses derived
from different explants with different (variable) potentials lead to different
proportions and forms of regeneration and morphology [11,30-32].

3.4. The Number of Somatic Embryos

The results presented in Table 4 show the superiority of the MS
medium regarding the number of embryos in both the dark and

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**Table 2:** Required days for forming indirect somatic embryos.

| Treatment | Leaves | Stems | Roots | Mean |
|-----------|--------|-------|-------|------|
| MS (DPM1) | 15     | 17    | 19    | 17<sup>a</sup> |
| B5 (DPM2) | 16     | 18    | 20    | 18<sup>b</sup> |
| LS (DPM3) | 15     | 18    | 19    | 17.33<sup>c</sup> |
| 5C01 (DPM4) | 15 | 17 | 18 | 16.66<sup>d</sup> |
| Mean  | 15.25<sup>a</sup> | 17.5<sup>a</sup> | 19<sup>a</sup> | 16<sup>a</sup> |

Means in the table are followed by different letters; the letters represent the order of the mean value which starts from the best value (a) and decline, respectively, according to the letter order.

**Table 3:** The percentages of indirect somatic embryo formation of the Digitalis purpurea explants in the dark and light conditions.

| Treatment | Leaves | Stems | Roots | Mean |
|-----------|--------|-------|-------|------|
| MS (DPM1) | 52.75  | 37.5  | 23.75 | 38<sup>a</sup> |
| B5 (DPM2) | 45.25  | 31.25 | 21.25 | 31.58<sup>b</sup> |
| LS (DPM3) | 49.5   | 32.5  | 21.25 | 34<sup>a</sup> |
| 5C01 (DPM4) | 45.75 | 26.25 | 18.75 | 30.25<sup>d</sup> |
| Mean  | 48.312<sup>a</sup> | 31.875<sup>a</sup> | 21.25<sup>a</sup> | 23.75<sup>a</sup> |

Means in the table are followed by different letters; the letters represent the order of the mean value which starts from the best value (a) and decline, respectively, according to the letter order.
Table 4: The number of indirect somatic embryos of the *Digitalis purpurea* explants in the dark and light conditions.

| Treatment  | Medium | Leaves | Stems | Roots | Mean | Leaves | Stems | Roots | Mean |
|------------|--------|--------|-------|-------|------|--------|-------|-------|------|
| MS (DPM1)  |        | 12.5   | 10.25 | 8.5   | 10.41| 14.25  | 11.25 | 9.75  | 11.75|
| B5 (DPM2)  |        | 7.25   | 8.25  | 5.5   | 7.00 | 8.5    | 8.75  | 6.75  | 8.00 |
| LS (DPM3)  |        | 9.75   | 9     | 6.25  | 8.33 | 11.25  | 9.75  | 7.5   | 9.50 |
| 5C01 (DPM4)|        | 8.25   | 8.5   | 5.25  | 7.33 | 9      | 8.75  | 6.25  | 8.00 |
| Mean       |        | 9.43   | 9     | 6.37  | 7.67 | 10.75  | 9.62  | 7.56  | 8.00 |

Means in the table are followed by different letters; the letters represent the order of the mean value which starts from the best value (a) and decline, respectively, according to the letter order.

light conditions compared to the rest of the other studied media, the superiority of the leaf explants compared to the stem and root explants, and the preference of light for inducing the number of indirect somatic embryos from the *D. purpurea* explants. These results agree with the findings of Mongomake *et al.* [33], showing that the number of buds formed per plant was significantly higher when incubated with an 8–16-h light cycle.

It was observed that the induction of some of the explants within the same medium was superior, as was the presence of some replicates without induction or within minimum limits. The lack of an appropriate number of induced somatic embryos in some of the explants or media was due to competition for mineral elements, the release of an inhibitory molecule, or the lack of a molecular signal [34].

In view of the superiority of the leaf explants regarding inducing calluses and indirect somatic embryos, the best hormonal combinations were selected for inducing indirect somatic embryos resulting from the tissue culture of *D. purpurea* leaves and the cell types isolated from them.

3.5. Selection of the Best Hormonal Combinations for the Rate of Callus Formation from *D. purpurea* Leaves

The callus stage is key in any system of the regeneration or micropropagation of indirect somatic embryos [15]. The quality of the callus plays an essential role in indirect plant regeneration, and compressed embryonic calluses were selected for successful *in vitro* regeneration [35]. The formation and development of indirect somatic embryos are also greatly influenced by the density of the culture (callus texture) [34], and the patterns of calluses differ depending on the type of nutrient media according to Gural *et al.* [29] on *Digitalis davisiana* Heywood.

The results presented in Table 5 show the selection of the best hormonal combinations for the rate of callus formation and maintenance in light and dark within the studied nutrient media. The concentrations of treatments for the selected growth regulators, where the combinations or treatments had higher rates of callus formation, were chosen compared to all the studied treatments in the presence of medium variables and concentrations of plant-growth regulators, taking into account the physical and morphological properties of the chosen callus. The results show that a concentration of (2 mg NAA + 1 mg Kin) L⁻¹ in the 5C01 medium was effective at a rate of 95%, and a concentration of (2 mg 2.4D + 1 mg BA) L⁻¹ in the MS medium induced callus formation rates of 85% and 90% in the dark and light, respectively, compared to the rest of the studied and selected combinations in the LS and B5 media and the presence of dark and light factors [Figure 1a-d]. Moreover, Table 5 also shows an improvement in the rate of callus induction in the MS, LS, and B5 media when comparing callus induction in dark and light conditions.

Table 5: The best-selected combinations for the rate of callus generation from leaves in the dark and light conditions for *Digitalis purpurea*.

| Treatment | Medium name | PGR concentration | Callus induction % |
|-----------|-------------|-------------------|--------------------|
| DPM1      | MS          | (2 mg 2.4D+1 mgBA) L⁻¹ | 85° | 90° |
| DPM2      | B5          | (2 mg 2.4D+1 mgBA) L⁻¹ | 70° | 75° |
| DPM3      | LS          | (2 mg NAA+2 mg BA) L⁻¹ | 75° | 85° |
| DPM4      | 5C01        | (2 mg NAA+1 mgKin) L⁻¹ | 95° | 95° |

The results of this study agree with the results of Besher [22] on the preference for the 5C01 medium for the induction and development of calluses of *H. aureus* and with Zhang *et al.* [15] on the advantage of the MS medium in inducing calluses of *D. hamiltonii* leaves.

3.6. Selection of the Best Hormonal Combinations for the Proportion of Indirect Somatic Embryo Formation

The results presented in Table 6 show that a concentration of (0.25 mg 2.4D + 2 mg BA) L⁻¹ in the MS medium resulted in the highest induction rate for indirect somatic embryos in the dark compared to the other studied treatments in the MS medium and all the treatments in the other nutrient media, followed by a concentration of (0.5 mg NAA + 2 mg BA) L⁻¹ in the LS medium. The studied treatments in the 5C01 and B5 media resulted in the lowest percentages of induction of indirect somatic embryos. Moreover, the results show that a concentration of (0.25 mg 2.4D + 2 mg BA) L⁻¹ in the MS medium achieved the highest induction rate for indirect somatic embryos (90%) in the light compared to the rest of the studied media and treatments.

The table also shows the discrepancy in the critical role of growth regulators according to the studied nutrient medium and the quality of the growth regulator. The effective superiority of one auxin over another within the nutrient media was attributed to the effective absorption and mobilization of the growth regulator or the rapid mobilization at the targeted sites [36]. The results of this study agree with those of Lijalem and Feyissa [37], which showed that different groups of growth regulators showed different responses in the formation of somatic embryos in *Securidaca longipedunculata*. They are also in agreement with the results of Gural *et al.* [29] on *Digitalis davisiana* Heywood, showing that the presence of significant differences in the renewal of shoots depending on the explant type, plant type, hormonal combination and components of the basal medium, and the timing and requirements of growth regulators for the growth of somatic embryos differs according to the studied species [38].

According to Tables 5 and 6, the explants in the light conditions exhibited embryonic calluses, distinguished by the asynchronous appearance of numerous structures, such as spherical, core, and
torpedoes. These structures were more pronounced in the stage of the third sub-culture while appearing in lower proportions in the explant calluses induced in the dark conditions in the fourth sub-culture [Figure 2a-d].

These results agree with those of Mongomake et al. [33] on the asynchronous formation of somatic embryos accompanied by several structures at different stages of development on the same structures of the callus. The subculture also allowed the calluses to gain mass in addition to somatic embryos [26]. There is a need to take into account the temporal estimation processes for the selection of the studied treatments to avoid obstacles to efficient somatic embryo induction resulting from the death of cells in contact with the agar surface or the formation of a brown callus (browning phenomenon), which limits the formation of somatic embryos, as high percentages of brown color reflect a decrease in physical embryonic development [38]. Furthermore, a callus or explant in contact with the agar surface shows a low response rate regarding the formation of somatic embryos. It comprises semi-dead cells due to poor breathing; the lack of oxygen leads to a poor supply of free energy [39,40]. These results agree with Chen et al. [17], showing that light is an important factor in improving regeneration efficiency in tissue cultures.

Indirect somatic embryos were developed in a plant in the dark after embryonic sprouts were transferred and grown in nutrient media according to the selected combinations presented in Table 6. Clear differences between the effect of the nutrient medium quality and the plant-growth regulators can be observed [Figure 3a-d].

On the other hand, Figure 3a-d shows the development of indirect somatic embryos in the light in a plant after they were transferred

Table 6: The best-selected combinations for the generation of indirect somatic embryos in the dark and light conditions for Digitalis purpurea.

| Treatment | Medium name | PGRs concentration | Indirect somatic embryos % |
|-----------|-------------|---------------------|----------------------------|
|           |             |                     | Dark | Light 16/8 |
| DPM1      | MS          | (0.25 mg 2.4D+2 mgBA) L⁻¹ | 52.75⁶ | 90⁶ |
| DPM2      | B5          | (0.5 mgNAA+2 mgBA) L⁻¹  | 45.25⁴ | 73.50⁴ |
| DPM3      | LS          | (0.5 mgNAA+2 mgBA) L⁻¹  | 49.50⁵ | 82.25⁵ |
| DPM4      | 5C01        | (1 mgNAA+1 mgKin) L⁻¹  | 45.75⁴ | 80⁴ |

Figure 1: The best cell lines selected from calli induced in the dark and light conditions.

Figure 2: Growth buds from indirect somatic embryos induced in the dark and light conditions.
to nutrient media containing hormonal combinations, according to Table 6. The effect of growth regulators that enhanced the nutrient medium was noted. In the presence of the light factor that fully promoted growth and development, compared to the embryos in Figure 3, the differences caused by the light factor in the development of indirect somatic embryos and their accurate reproduction can be observed.

4. CONCLUSIONS

Based on the present experimental investigation, the following findings have been drawn; the 5C01 and MS media were the optimum media for callus induction in D. purpurea, while the MS and LS media were preferred for indirect somatic embryogenesis formation and induction. It has been also observed that the basal nutrient medium and the type and concentration of growth regulators were critical and essential factors for callus and indirect somatic embryogenesis induction. Moreover, the type of vegetative explant and light were essential factors in the induction of indirect somatic embryos from calluses.

5. AUTHORS’ CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Mohammed Ahmed Al-Oqab. The first draft of the manuscript was written by Mohammed Ahmed Al-Oqab, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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7. DATA AVAILABILITY STATEMENT

The data presented in this study are available upon request from the corresponding author.

8. CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

9. ETHICAL APPROVAL

This research did not involve experiments with human or animal participants.

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REFERENCES

1. Olmstead RG. Whatever happened to the Scrophulariaceae. Fremontia 2002;30:13-22.
2. Council of Europe. European Pharmacopoeia 8th ed. Europe: European Pharmacopoeia; 2014.
3. Withering W. An Account of the Foxglove, and Some of its Medical Uses. Cambridge: Cambridge University Press; 2014.
4. Goldthorp WO. An Account of the foxglove. BMJ 2009;338:b2189.
5. Chen S, Khusial T, Patel D, Singh S, Yakubova T, Wang A, et al. Digoxin use in modern medicine. US Pharm 2015;40:44-8.
6. Stenkvist B, Bengtsson E, Dahlqvist B, Eriksson O, Jarlkrans T, Nordin B. Cardiac glycosides and breast cancer, revisited. N Engl J Med 1982;306:484.
7. Platz EA, Yegnasubramanian S, Liu JO, Chong CR, Shim JS, Kentfield SA, et al. A novel two-stage, transdisciplinary study identifies digoxin as a possible drug for prostate cancer treatment. Cancer Discov 2011;1:68-77.
8. Cho J, Lee YJ, Kim JH, Kim S il, Kim SS, Choi BS, et al. Antiviral activity of digoxin and ouabain against SARS-CoV-2 infection and its implication for COVID-19. Sci Rep 2020;10:16200.
9. Verma SK, Das AK, Cingoz GS, Gurel E. In vitro culture of digitalis L. (Foxglove) and the production of cardenolides: An up-to-date review. Ind Crops Prod 2016;94:20-51.
10. Probert R, Adams J, Coneybeer J, Crawford A, Hay F. Seed quality for conservation is critically affected by pre-storage factors. Aust J Bot 2007;55:326-35.
11. Mandal J, Laxminarayana U. Indirect shoot organogenesis from leaf explants of Adhatoda vasica Nees. Springerplus 2014;3:648.
12. Jain SM, Gupta PK. Protocol for Somatic Embryogenesis in Woody
Plants. Netherlands, Dordrecht: Springer; 2005.

13. Mujib A, Samaj J. Somatic Embryogenesis. Vol. 2. Berlin, Heidelberg: Springer; 2006.

14. Lema-Rumińska J, Goncerzewicz K, Gabriel M. Influence of abscisic acid and sucrose on somatic embryogenesis in cactus Copiapoa Teniusissima ritt. Forma mostruosa. Sci World J 2013;2013:513985.

15. Zang Q, Zhou L, Zhuge F, Yang H, Wang X, Lin X. Callus induction and regeneration via shoot tips of Dendrocalamus hamiltonii. Springerplus 2016;5:1799.

16. Hallioglu K, Aydin M. Efficient regeneration system from rye leaf base segments. Springerplus 2016;5:2005.

17. Chen YM, Huang JZ, Hou TW, Pan IC. Effects of light intensity and plant growth regulators on callus proliferation and shoot regeneration in the ornamental succulent Haworthia. Bot Stud 2019;60:10.

18. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plantarum 1962;15:473-97.

19. Gamborg OL, Miller RA, Ojima K. Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res 1968;50:151-8.

20. Linsmaier EM, Skoog F. Organic growth factor requirements of tobacco tissue cultures. Physiol Plant 1965;18:100-27.

21. Vollosovich A, Puchinina T, Nukolaeva L. Optimization of the composition of macrosalts for tissue culture of Rauwolfia serpentina Benth. Rastitel Resursy 1979;5:516-26.

22. Besher SH. Effect of nutritive medium and change in gene expression of tropinin reductase I and tropizin reductase II on the growth dynamic and production of hydroxyamine and scopolamine in callus culture of Hyoscyamus niger L. Syrian J Agric Res 2022;8:320-38.

23. Verma SK, Das AK, Cingoz GS, Uslu E, Gurel E. Influence of nutrient media on callus induction, somatic embryogenesis and plant regeneration in selected Turkish Crocus species. Biotechnol Rep 2016;10:66-74.

24. Dou H, Niu G, Gu M, Masabni JG. Effects of light quality on root formation from root-derived callus of Oncidium “Gower Ramsey”. Plant Cell Tissue Organ Cult 2004;77:107-9.

25. Krishnan SR, Priya AM, Ramesh M. Rapid regeneration and ploidy stability of “cv IR36” indica rice (Oryza sativa. L) confers efficient protocol for in vitro callus organogenesis and Agrobacterium tumefaciens mediated transformation. Bot Stud 2013;54:47.

26. Chen JT, Chang WC. Efficient plant regeneration through somatic embryogenesis from callus cultures of Oncidium (Orchidaceae). Plant Sci 2000;160:87-93.

27. Wu IF, Chen JT, Chang WC. Effects of auxins and cytokinins on embryo formation from root-derived callus of Oncidium “Gower Ramsey”. Plant Cell Tissue Organ Cult 2004;77:107-9.

28. Gurel E, Yucesan B, Aglic E, Gurel S, Verma SK, Sokmen M, et al. Regulation and cardiotonic glycoside production in digitalis daviana heywood (Alanya foxglove). Plant Cell Tissue Organ Cult 2011;104:217-25.

29. Erisen S, Yorgancilar M, Atalay E, Babaoglu M. Prolific shoot regeneration of Astragalus cariensis Boiss. Plant Cell Tissue Organ Cult 2010;100:229-33.

30. Lin GZ, Zhao XM, Hong SK, Lian YJ. Somatic embryogenesis and shoot organogenesis in the medicinal plant Pulsatilla koreana Nakai. Plant Cell Tissue Organ Cult 2011;106:93-103.

31. Kumar S, Kashyap M, Sharma DR. In vitro regeneration and bulblet growth from lily bulbscale explants as affected by retardants, sucrose and irradiance. Biol Plant 2005;49:629-32.

32. Mongomake K, Doupous O, Khatabi B, Fondong VN. Somatic embryogenesis and plant regeneration of cassava (Manihot esculenta Crantz) landraces from Cameroon. Springerplus 2015;4:477.

33. Moon HK, Kim YW, Hong YP, Park SY. Improvement of somatic embryogenesis and plantlet conversion in Oplopanax elatus, an endangered medicinal woody plant. Springerplus 2013;2:428.

34. Chaudhary J, Danu PK. Induction of somatic embryos in cultures of Asparagus racemosus Willd: An endangered medicinally important plant. Bull Nall Res Cent 2019;43:1-15.

35. Karun A, Sirili EA, Radha E, Parthsarathya VA. Somatic embryogenesis and plantlet regeneration from leaf and inflorescence explants of arecanut (Areca catechu L.). Curr Sci 2004;86: 1623-8.

36. Lijalem T, Feyissa T. In vitro propagation of Securidaca longipedunculata (Fresen) from shoot tip: An endangered medicinal plant. J Genet Eng Biotechnol 2020;18:3.

37. Shen HJ, Chen JT, Chung HH, Chang WC. Plant regeneration via direct somatic embryogenesis from leaf explants of Tolumnia Louise Elmore “Elsa”. Bot Stud 2018;59:1.

38. Bhusare BP, John CK, Bhatt VP, Nikam TD. In vitro propagation of Digitalis lanata Ehrh. Through direct shoot regeneration a source of cardiotonic glycosides. Ind Crops Prod 2018;121:313-9.

39. Hasan M, Bano A, Hassan S, Iqbal J, Awan UF, Rongji D, et al. Enhancement of rice growth and production of growth-promoting phytohormones by inoculation with Rhizobium and other rhizobacteria. World Appl Sci J 2014;31:173-43.

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