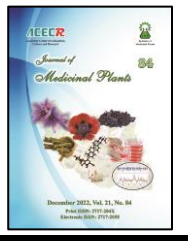
J. Med. Plants 2022; 21(84): 50-64

Institute of Medicinal Plants

Journal of Medicinal Plants

Journal homepage: www.jmp.ir



Research Article

Influence of different *Agrobacterium rhizogenes* strains on hairy roots induction and secondary metabolites production in *Datura innoxia* Mill.

Parisa Naderian[1], Nasrin Moshtaghi[2,*], Abdolreza Bagheri[2], Saeid Malekzadeh-Shafaroudi[2]

[1] *MSc of Agricultural Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran*

[2] *Department of Biotechnology and Plant Breeding, Ferdowsi University of Mashhad, Mashhad, Iran*

ARTICLE INFO

Keywords:
Agrobacterium rhizogenes
Culture
Hairy root
In vitro
Hyoscyamine

ABSTRACT

Background: *Datura innoxia* Mill. is one of the medicinal plant which can produce tropan alkaloids such as hyoscyamine and scopolamine with analgesic and antiasthmatic activities. **Objective:** In this research, the effect of various factors such as culture media and bacteria strain were studied on hairy roots induction and secondary metabolites production in *Datura innoxia*. **Methods:** Strains A4, A13, R1000, MSU, 15834, 2656 and 11325 of *Agrobacterium rhizogenes* with two different concentrations (OD = 0.5, 1) used for co-cultivation of leaf and stem explants prepared from 1-3-month-old and 5-7-month-old plants to optimize the production of hairy roots and then 6 different media were used for establishment and growth of hairy roots. **Results**: The results of data analysis showed that A4 strain with 86.6 % hairy root production was the best strain than others. Also, dark conditions, younger explants and higher concentration of bacteria caused the highest amount of induction of hairy roots. ½ MS medium was recognized as the best medium for the growth of hairy roots. The results of HPLC analysis also showed that the level of hyoscyamine in hairy roots was lower than that of normal roots which, of course, can be compensated by the higher growth of hairy roots. **Conclusion**: The appearance and growth of hairy roots depends on various factors such as bacterial strain, culture medium, explant and its age and other factors that need to be optimized at the beginning of any research.

1. Introduction

Datura innoxia Mill. belongs to Solanaceae family and is well-known as the angel's trumpet or thorn apple in the world. This plant is a perennial and dicotyledonous which can grow in the form of bushes in warm climates [1]. *Datura* species produce a wide spectrum of tropane alkaloids, whose chemical synthesis is difficult and uneconomical [2]. The use of hairy roots can be a good alternative for the production of specialized metabolites on a large scale [3].

The utilization of hairy root culture is a relatively new approach that has received increasing attention in recent years. However, in

Abbreviations: MS Medium, Murashige and Skoog Medium; B5, Gamborg et al. Medium; YMB, Yeast Maltose Broth

*Corresponding author: moshtaghi@um.ac.ir

doi: 10.52547/jmp.21.84.50

Received 28 August 2022; Received in revised form 23 November 2022; Accepted 26 November 2022

the mid-1980s, evidence of the potential of hairy roots was obtained through a series of studies that focused on the alkaloid production [4]. *Agrobacterium rhizogenes* transmits the part of a root-promoting plasmid to the plant cell and causes hairy root production [5].

The hairy roots are longer and more branched than normal roots, also they have no geotropism and apical dominance. They can grow rapidly in the absence of external plant growth regulators. Since the production of secondary metabolites is usually greater in differentiated tissues, hairy roots provide an alternative technique to the use of cell suspension cultures [4, 6].

Most studies using hairy roots have been associated with the production of valuable medicinal compounds. Hairy roots have also been used to isolate new natural products with medicinal activity, regenerate transgenic plants with beneficial traits and studying the regulatory pathways of secondary metabolism [7, 8]. The phenolic acid compounds in the hairy roots of the bitter melon plant *Momordica charantia* [9] and the medicinal compound sanguinarine in hairy roots of *Macleaya cordata* plant [10] showed enhanced accumulation of metabolites compared to natural roots.

Recombinant proteins can also be produced in transgenic hairy roots, so they have a great potential for the pharmaceutical industry. The production of M12 monoclonal antibody with good efficiency was done by Hakkinen et al. (2014) in hairy roots of tobacco plant [11]. Another research is the production of human gastric lipase in the hairy roots of *Brassica napus* plant, this enzyme has therapeutic properties and is used to compensate the deficiency of pancreatic enzymes. It contributes to fatty acid release from ingested triglycerides [12]. There are various researches about molecular farming through production of recombinant proteins in hairy root culture [13]. Due to the importance of hairy roots, this study aimed to investigate the effect of different strains of *A. rhizogenes* and other factors on production and growth of hairy roots.

2. Materials and Methods

2.1. Induction of hairy roots in diploid plants

The leaves and nodes of 6–8 weeks old plants of *Datura innoxia* (with herbarium code 11257, FUMH), grown in the greenhouse, were used as explants. The explants were sterilized with sodium hypochlorite 2 % for 15 minutes and then washed with sterile water three times. In this experiment, strains A4, A13, R1000, MSU, 15834, 2656 and 11325 of *Agrobacterium rhizogenes* were used in YMB culture medium containing 100 mg/L of rifampicin antibiotic. Sterile leaf explants were placed upside down in petri dishes containing solid MS [14] and B5 [15] media in a laminar air flow compartment and inoculated with bacterial suspension using a 100 µl insulin syringe.

Stinging was performed mostly on the veins and marginal parts of leaf explants. 500 mg/L cefotaxime was used to remove the bacteria. About two weeks after inoculation, the first transgenic hairy roots appeared at the inoculation site. When the length of the emerging roots reaches about 2-3 cm, the roots are cut and transferred in to the flasks containing 30 ml of $^1/_2$ MS liquid culture medium containing 300 mg/L of cefotaxime and pH 5.5. The flasks were then placed in the dark on a rotary shaker at 1 g. To confirm the transgenic nature of hairy roots, a specific PCR reaction was performed for the amplification of *rol*C gene. The primers used to amplify the *rol*C gene are: Forward primer with 5′-CTCCTGACATCAAACTCGTC-3′ sequence and reverse primer with 5′-TGCTTCGAGTTATGGGTACA-3′ sequence.

[DOR: 20.1001.1.2717204.2022.21.84.5.7]
[DOI: 10.52547/jmp.21.84.50]

2.2. Investigation of various factors on the appearance of hairy roots

The factors were studied for the appearance of hairy roots, described below. The variables were examined in separate experiments. All strains A4, A13, R1000, MSU, 15834, 2656 and 11325 of *A. rhizogenes* with two different concentrations (OD = 0.5, 1) used for co-cultivation of leaf and stem explants prepared from 1–3-month-old and 5–7-month-old plants. The explants were located on MS and B5 media. In the next step, MS and B5 media containing cefotaxime were used to culture the explants. In this way, the samples cultured on MS culture medium were transferred to both MS and B5 media containing cefotaxime. The culture medium contained cefotaxime until the appearance of hairy roots. The same situation was done for explants cultured in B5 medium. To measure the effect of light and darkness on the appearance of hairy roots, the samples were exposed to both dark and light conditions. The percentage of root appearance was calculated by dividing the number of inoculated explants with roots to all the inoculated explants.

2.3. Investigation of factors affecting hairy root growth

Among the factors affecting the growth of hairy roots, we studied factors such as bacterial strain, size of hairy roots at the time of transferring to the liquid culture medium, type of liquid culture medium and storage conditions of culture medium (shaking and no shaking). In this regard, an experiment was performed to investigate the effect of bacterial strains (strains A4, A13, MSU, 15834 which produced a high percentage of hairy roots) and the size of hairy roots at the time of transferring to the liquid culture medium (2 and 5-7 cm). Experiments related to the effect of liquid culture medium and their storage conditions on the production of hairy root biomass were performed as a factorial experiment in a completely randomized design for two strains of A4 and A13 that grew well compared to other strains.

Six liquid culture media of MS, $^1/_2$ MS, $^1/_4$ MS, B5, $^1/_2$ B5 and $^1/_4$ B5 with sucrose concentration of 30 g/L, in 100 ml vials containing 25 ml of culture medium containing 300 mg/L cefotaxime, each with 5 replications and under two conditions of shaker and without shaker were used. After twelve weeks, when the roots grew well, the roots were freeze-dried, weighed and stored for alkaloid extraction.

2.4. Extraction and measurement of hyoscyamine alkaloid

Freeze-dried samples were powdered in mortar and used for alkaloid extraction. 300 mg of each powder was weighed and put into the 50 ml Falcon tubes. 10 ml of 96 % ethanol was added to each of them and then were placed on a shaker for 48 hours with a gentle motion at 25 °C. After that, the ethanol was evaporated by vacuum evaporation and 5 ml of 5 % sulfuric acid was added to each and placed in a gentle rotation for 16 hours at 25 °C. Then, in 3 steps, 3 ml of chloroform was added to the mixture in each time, and the chloroform precipitate containing colorants was removed.

The precipitated acidic solution was filtered and adjusted to pH 10 using NaOH of 10 N. Then, in 3 steps, 3 ml of chloroform was added to the remaining solution and the chloroform precipitate containing the alkaloids was removed. The evaporated solvent was dissolved in the mobile phase and 20 µl of each was injected into the device after passing through a syringe filter. The HPLC device was from the German company Zorbox, with a two-pump system, equipped with a S2600 photodiode

[DOR: 20.1001.1.2717204.2022.21.84.5.7]
[DOI: 10.52547/jmp.21.84.50]

detector with a loop volume of 20 µl, and the EZ Chrom Elite software program with integration capabilities .The constant phase used was C_{18} column with an inner diameter of 4.6 mm and a length of 250 mm, and water: methanol: acetonitrile with a ratio of 80:10:10 was used as the mobile phase. The wavelength used was also 254 nm. The mobile flow velocity in this experiment was considered to be 1 ml/min. Solution of pure hyoscyamine powder in methanol was used to prepare 2500 ppm of hyoscyamine as standard.

2.5. Data analysis

The variables were examined in separate experiments as completely randomized design with five replications for each treatment. Statistical analyzes were done after data normalization. All data containing the percentage of hairy root appearance normalized by Arcsin equation and then analysed by JMP ver 4 software. Mean comparisons were done using LSD test and the curves were drawn by Excel software.

3. Results

3.1. Production of transgenic hairy roots

Transgenic hairy roots began to appear from the inoculation site about two weeks after inoculation of leaf with *A. rhizogenes* A4, and this process continued for 15 days after that. The exit site of transgenic hairy roots from the inoculum was showed in Fig. 1. When the size of the emerging roots reached to 5-7 cm, the roots were cut off and transferred to B5 liquid culture medium (Fig. 1C). $^1/_2$ MS liquid culture medium was used for establishing the hairy roots and decreasing the bacterial contamination.

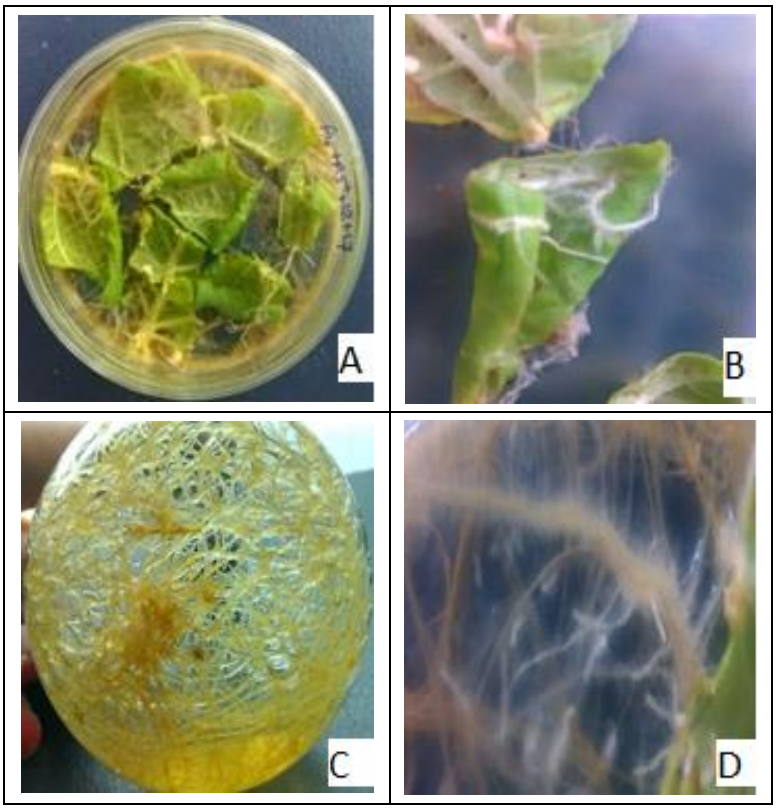


Fig. 1. Hairy roots appearance from the inoculation site after co-cultivation with A4 strain (A and B), hairy root growth in B5 liquid culture medium (C) and the hairy apperance of the roots (D)

The transgenic hairy roots had a high growth rate, hairy and branched appearance (Fig. 1). Also they were able to produce callus, mainly due to the presence of an internal source of the auxin hormone [16] while normal roots had less growth and branching compared to the transgenic hairy roots.

The amount of transgenic hairy roots after two months of growth in $^{1}/_{2}$MS liquid culture medium increased significantly, which made it possible to conduct a study to evaluate the stability and growth rate.

Different clones of hairy roots had significant differences, so the clones with slow growth were removed. Therefore, in this study, from the available clones, the clones that had a better growth rate were selected for the following experiments.

3.2. Appearance of hairy roots

3.2.1. The effect of different bacterial strains

By applying 7 bacterial strains (A4, A13, 15834, 2656, MSU, R1000 and 11325) on explants, a significant difference was observed among the strains in terms of the percentage of hairy root appearance. All of these bacterial strains except 11325 strain were able to produce hairy roots. All strains of A4, A13, 15834, R1000 and MSU were able to produce hairy roots after 15 to 20 days of inoculation, while in explants inoculated by 2656 strain, transgenic hairy roots began to appear after four weeks.

Strain 11325 did not produce any hairy roots and only callus appeared at the inoculation site. The results of data analysis showed a significant difference between different strains of bacteria in terms of the percentage of hairy root production. The percentage of hairy root appearance was different in bacterial strains as it was in A4 (86.66 %), A13 (66.66 %), 15834 (75.95 %), 2656 (15.38 %), MSU (45 %), R1000 (28.45 %) and 11325 (0 %) (Fig. 2).

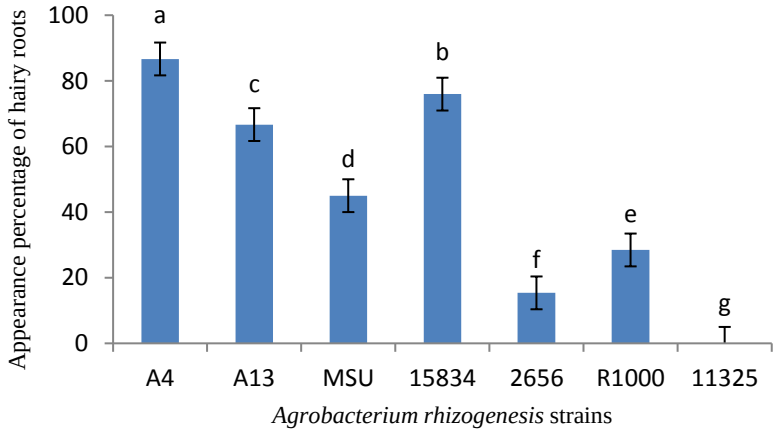


Fig. 2. The effect of bacterial strain on the percentage of root appearance. (The error bars are SE and the same letters show no significant difference based on LSD test at 0.05 significant level)

Therefore, A4 strain in the same conditions showed the highest percentage of hairy root production compared to other bacterial strains. They also had a higher rate of growth. In strain 11325, only callus production was observed at the inoculation site and no hairy roots appeared (Fig. 3). Strain 2656 also produced very delicate and small roots after one month of inoculation, which died after three days.

These results showed that the kind of bacterial strain is affected the production of hairy roots which depends on their pathogenicity.

3.2.2. The effect of A4 bacterial concentration

This experiment was performed using A4 bacteria with two concentrations of OD (0.5 and 1) in five replicates. The results showed a significant difference between the two ODs. Approximately, 56% and 76% of root appearance were observed at OD 0.5 and 1 respectively (Fig. 4).

Fig. 3. Calli formed on the explant due to inoculation with strain 11325

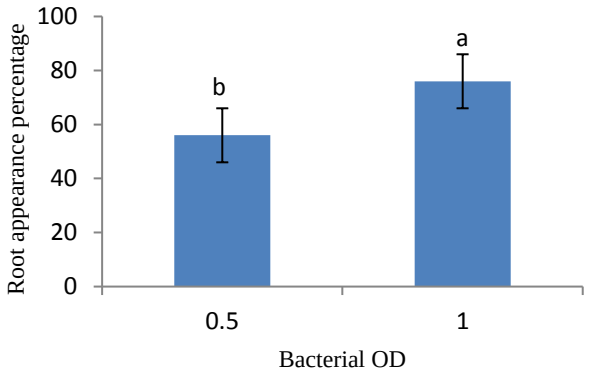


Fig. 4. The effect of bacterial OD of A4 strain on root appearance percentage. (The error bars are SE and the same letters show no significant difference based on LSD test at 0.05 significant level)

3.2.3. Co-culturing

This experiment was performed to determine the most suitable medium for co-culturing with bacteria and the appearance of hairy roots. Two culture media included B5 and MS were compared in Table 1.

The results showed that MS-MS and MS-B5 were the most suitable media with 72% of hairy root appearance. But between the two best, MS-MS culture medium seems to be more desirable for preventing high bacterial contamination in explants. High concentration of inorganic salts in MS medium probably prevents the high growth of bacteria. The rapid growth of bacteria at the same time in B5 culture medium compared to MS is quite evident in Fig. 5.

3.2.4. Age of explants for inoculation

The age of seedlings from which explants were prepared, also had an effect on the rate of hairy root appearance. 4–6-week-old seedlings inoculated with bacteria had the highest percentage of root appearance in the shortest time, i.e. two weeks, and the appearance of hairy roots decreased when the explants obtained from 5-7 months old plants.

Table 1. Percentage of hairy root appearance in different culture media

Co-culturing medium	Culture medium containing cefotaxim for root appearance	Percentage of hairy root appearance
MS	MS	72[a]
MS	B5	72[a]
B5	MS	64[b]
B5	B5	52[c]

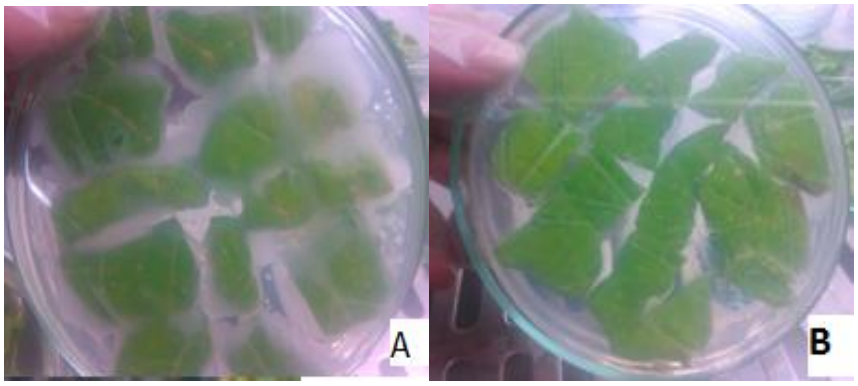


Fig. 5. Bacterial growth rate around explants in the same time in two culture media: B5 (A) and MS (B)

3.2.5. Types of explants

Leaf explants had the highest ability to produce transgenic hairy roots after inoculation with A4 strain and the time was also shorter than the other ones. The first hairy roots appeared from leaf explants, 2 weeks after inoculation, but the first hairy roots appeared in stem explants about one month after inoculation. As a result, leaf explants appear to be more suitable for inoculation.

3.2.6. The effect of light and darkness

The results showed that the appearance and growth of hairy roots in dark conditions was greater (4-5 days earlier) than in the light conditions. Also, more growth and density of hairy roots was observed in dark conditions (Fig. 6).

3.2. Confirmation of the transgenic nature of hairy roots

The results of PCR analysis also confirmed the presence of a 612 bp fragment related to the rolC gene in hairy roots (Fig. 7). The PCR with the same primers and DNA of natural roots did not produce any bands.

3.3. Growth of hairy roots

3.3.1. Effect of bacterial strains

There was a significant difference in growth of hairy roots which were obtained by inoculation of strains A4, A13, 15834 and MSU in ½ MS liquid culture medium. Obtained hairy roots using A4 strain, had the highest growth rate but the hairy roots using MSU strain, had the lowest growth compared to other clones (Fig. 8). Meanwhile, hairy roots using strains A13 and A4 showed better growth than those inoculated by MSU and 15834, so the experiments continued with focus on these two strains.

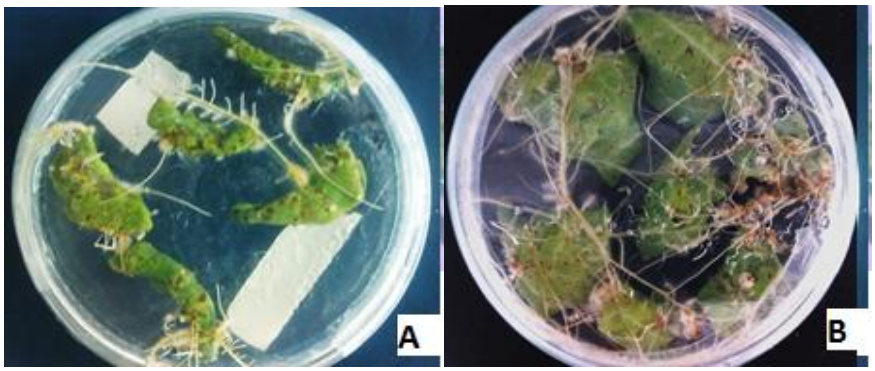


Fig. 6. The rate of appearance and growth of hairy roots in both light (A) and dark (B) conditions after the same period of one month

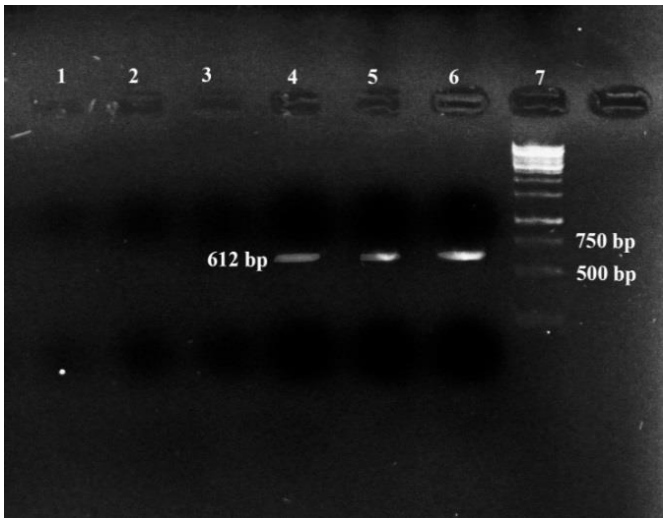


Fig. 7. Confirmation of transgenic natures of hairy roots by PCR, line 1 is a negative control, line 2 is also a PCR product of MS liquid culture medium, line 3 is natural root showed no band, lines 4 and 5 are related to the mplified *rol*C gene in transgenic hairy root, amplified *rol*C gene belonging to *Agrobacterium rhizogenes* A4 and line7 is the size marker (Ladder 1 kb, fermentase)

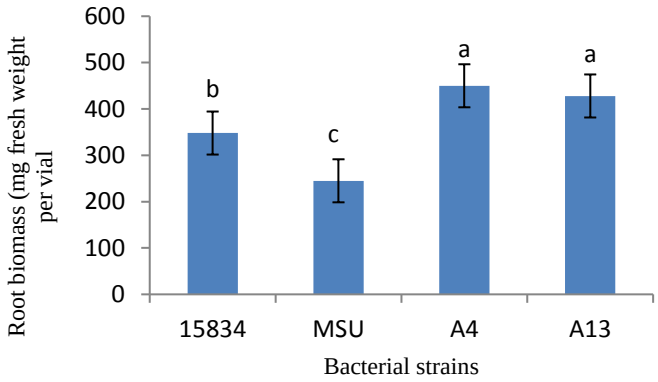


Fig. 8. Root biomass (mg fresh weight per vial) after 1 month in ½ MS medium (The error bars are SE and the same letters show no significant difference based on LSD test at 0.05 significant level)

In order to determine the growth of hairy root, a factorial experiment in a completely randomized design including two factors of bacterial strains (A4 and A13, which had better growth than other strains) and times (2, 4, 6, 8 and 10 weeks of growth) was performed with 5 replications for each treatment in ½ MS culture medium and the growth trend was plotted (Fig. 9).

In general, it was concluded that the growth rate is different at various times, culture media and strains.

There was a difference in the morphology of roots from different strains. The hairy roots from A4 strain were white, branched and longer, also they had high density, fast growing and a callous state. In A13 strain, white roots, short hairs with high density, more branching than A4 and little callus status were observed. Strain 15834 produced roots with many branches and fewer hairs than strains A13 and A4. Slow and weak growth was another characteristic of transgenic roots obtained from these strains. The MSU strain also produced delicate roots with thin, short hairs that grew little and died after a while.

3.3.2. The effect of hairy root size during transferring to culture medium

To achieve a suitable mass of hairy roots, they were separated from the leaves and transferred to a new culture medium. When the size of the hairy roots reached 5-7 cm and almost covered the surface of the petri, it was a good time to separate them from the leaves. It seemed that earlier transferring (2-3 cm) caused no growth and damage to the hairy roots. Bacterial contamination will also occur due to the presence of possible bacteria.

3.3.3. Influence of culture medium

Reports indicate that the rate of hairy root growth varies in different culture media. In this regard, an experiment was performed in MS, ½ MS, ¼ MS, B5, 5 B5, ¼ B5 culture media with two bacterial strains (A4 and A13) and 5 replications for each treatment.

In general, liquid culture media ½ MS, ¼ MS and B5 showed the highest growth rate, which may be due to the low concentration of salts in these media. Also, as can be seen, the effect of culture medium on the growth rate of hairy roots is different among different strains and in general, A4 strain has better growth than A13, but in this situation, ¼ MS did not show a significant difference (Fig. 10).

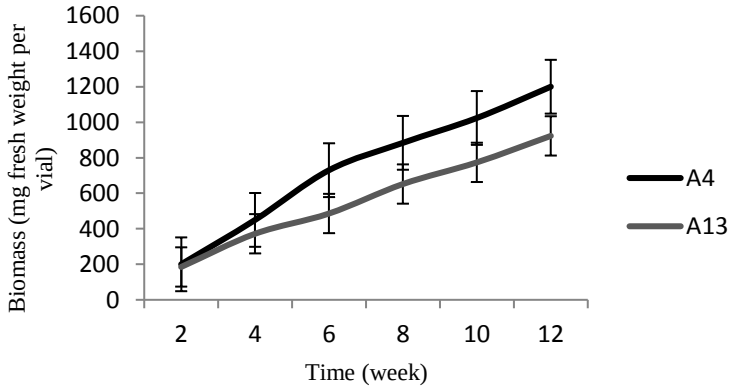


Fig. 9. Growth curve of hairy roots obtained from two bacterial strains at different times in ½ MS culture medium. (The error bars are SE)

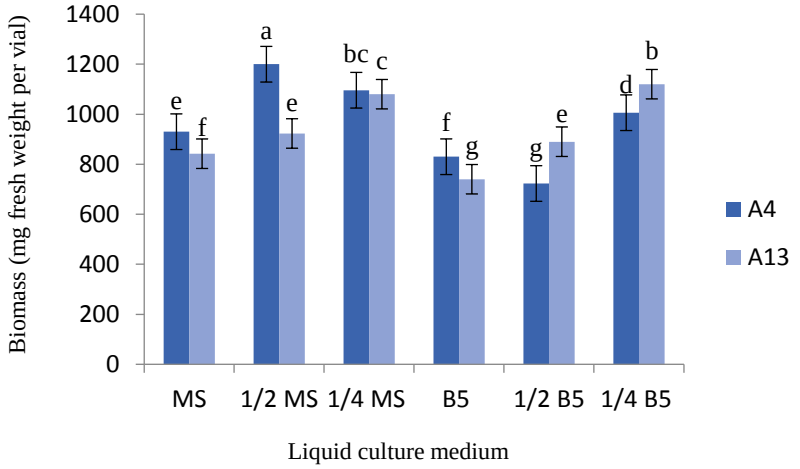


Fig. 10. The effect of different culture media on hairy root biomass from two strains after 3 months (The error bars are SE and the same letters show no significant difference based on LSD test at 0.05 significant level)

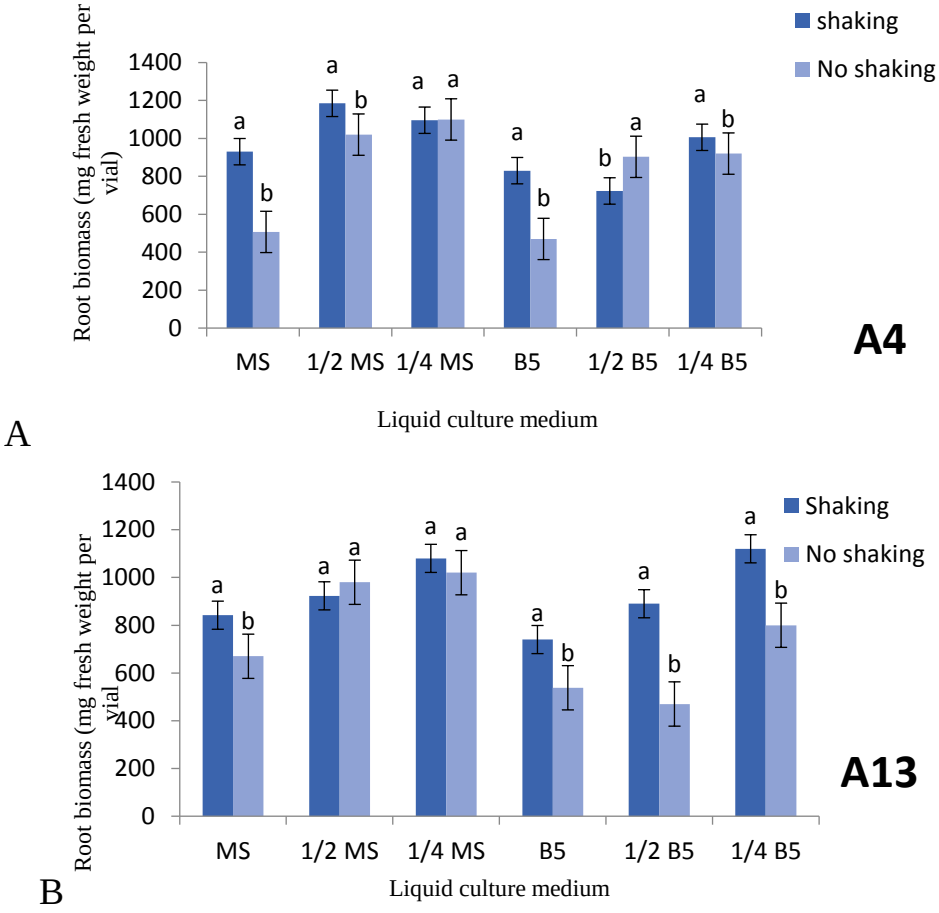


Fig. 11. The effect of culture medium and shaking condition on hairy root biomass of A4 strain (A) and A13 (B), (average comparison was done for each environment separately) (The error bars are SE and the same letters show no significant difference based on LSD test at 0.05 significant level)

3.3.4. Effect of shaker and no shaking conditions

By analyzing the data from the conditions considered for the culture medium of hairy root growth (on shaker and without shaker), it was concluded that shaking is more suitable (Fig. 11 A and B). After 2.5 months, the biomass of 961.66 and 932.5 mg of fresh weight were recorded for hairy roots obtained for A4 and A13 strains, respectively on shaking condition and 820 and 746 mg in without shaking.

3.4. Comparison of hyoscyamine content

In general, natural roots had more hyoscyamine than hairy roots from diploid plants. Also, hairy roots grown in MS medium produced more hyoscyamine than hairy roots grown in ½ MS and B5 medium (Fig. 12). The low production of hyoscyamine may be due to the conversion of hyoscyamine to scopolamine.

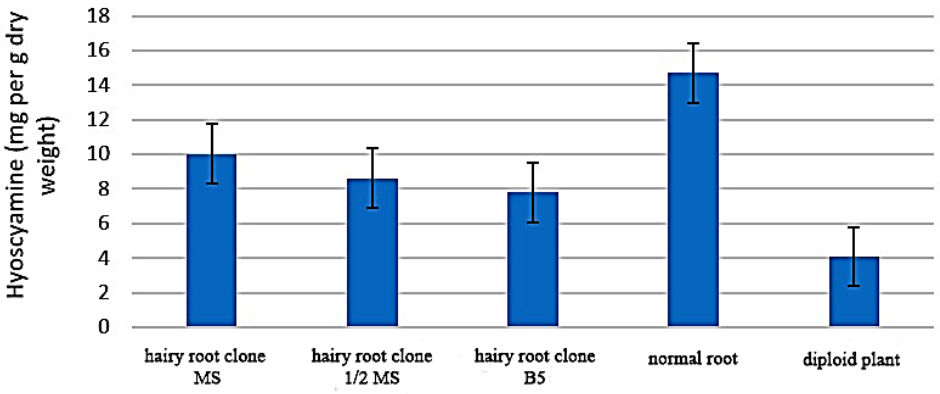


Fig. 12. Hyoscyamine content in different clones (The error bars are SE)

4. Discussion

In this experiment, different factors for appearance and growth of hairy root culture in *Datura innoxia* were examined and the results showed that different strains of bacteria, kind of explants, age of explants and culture medium affected the appearance and the growth of hairy roots. Differences in biomass production and secondary metabolite production in different clones of hairy roots have been observed previously in different studies [17, 18, 19, 20, 21]. Due to random insertion of T-DNA, different doses and different T-DNA expression in the host transgenic cell genome, significant differences among clones can be justified and it seems that the selection of superior clones to increase production, is very important [22]. Also the various strains of *Agrobacterium* have different gene arrangement in their root inducing plasmid so they have different capacity for induction of roots [13]. Sathasivam et al. [23] observed a significant difference among the six strains in terms of hairy root production. In their study, the transformation efficiency of various *A. rhizogenes* strains (ATCC 13333, ATCC 15834, A4, R1000, R1200, and R1601) was examined in *Ocimum basilicum*. Among the strains, the R1601 was the best for high transformation efficiency of 94 %. Plant species can affected considerably the selection of a suitable strain of *Agrobacterium* to obtain transformed hairy roots, so it needs to be investigated experimentally [24]. In *Catharanthus roseus*, a significant difference in the rate of hairy root appearance was observed among the five strains of A4, A13, 15834, R1000 and MSU [25]. Also in *Datura stramonium* plant, a significant difference was observed between A4 and LBA9402 strains for hairy root production (80 % and 40% respectively). Also, the growth rate of transgenic

[DOR: 20.1001.1.2717204.2022.21.84.5.7]
[DOI: 10.52547/jmp.21.84.50]

roots with A4 was higher than LBA9402 [20]. This phenomenon was observed in other study [26].

Different explants with various ages have different capacity for producing of hairy roots because they have varied contents of indigenous hormones which affected the hairy root production [3]. In *Datura* 3-month-old plants was better than older for hairy root production. The time for hairy root appearance was reduced to two weeks in younger seedling [27].

Co-culturing media was also affected the production of hairy roots as if we use MS medium, the percentage of root production will increase. MS medium have more inorganic salts and lower organic material in comparison with B5 medium [14, 15]. Therefore the growth of bacteria in MS medium was lower than B5 medium and the contamination with bacteria was lower. So the chance of survival explant will increase for producing of hairy roots.

The appearance and growth of hairy roots was also better in dark than light. When the roots are directly exposed to light, there is a reduction in root length [28]. Light can stimulate the root growth by producing sugars and auxin in aerial parts of whole plant and also red and blue light exhibit a positive effect on root elongation in comparison with darkness [29]. However, adding of sucrose to the culture medium can sometimes reverse this effect [30].

Higher concentration of hyoscyamine in MS medium can be due to higher concentrations of nutrients, especially ammonium and nitrate in the culture medium [31]. Although the production of hyoscyamine alkaloid was lower in transgenic hairy roots than in normal roots, but due to the high growth rate of transgenic hairy roots, the total production of hyoscyamine in them is higher. Another hypothesis is that the most of produced hyoscyamine was consumed for production of scopolamine. In a study by Moyano [32] on *Datura*, Suwon et al. [33] on Hyoscyamus and Farsi et al. [20], similar results were obtained. There are different repots about the content of metabolites in hairy root clones from different plants [34, 35]. If we could take a hairy root clone with high production of hyoscyamine and scopolamine content, it can be a good alternative for production of specialized metabolites in pharmaceutical industry. Although other alternatives such as polyploidization and metabolic engineering can be used for increasing the metabolites in medicinal plants [36].

5. Conclusion

Root appearance and its growth can be affected by *A. rhizogenes* strain, kind of explant and lower age of explants. Also culture media and the light can impact on root appearance and its growth. Culture media especially MS and ½ MS with low concentration of MS salts determined as the optimized medium for better growth of hairy roots. Although the hyoscyamine content was lower in hairy root clones, the growth of hairy roots was more than normal roots in a short time, so using hairy roots for producing specialized metabolites can be a good alternative for the whole plant.

Author contributions

Conceptualization, NM; Formal analysis, PN; Investigation, PN and NM; Methodology, PN; Supervision, NM and AB; Writing - review & editing, NM, SM.

Conflict of interests

The authors declare that there is no conflict of interest.

Acknowledgements

We thank from FUM for its financial support and instruments.

[DOR: 20.1001.1.2717204.2022.21.84.5.7]
[DOI: 10.52547/jmp.21.84.50]

References

1. Mardare (Balusescu) G, Malutan T and Lazar L. Determination of scopolamine by gas chromatography from different parts of the *Datura innoxia* biomass. *Rev. Chim*. 2020; 71(7): 126-136. doi: 10.37358/RC.20.7.8230.

2. Lounasmaa M and Tamminen T. The Tropane Alkaloids. In Brossi A (ed.), The Alkaloids, New York: Academic; 1993: 1-113.

3. Li C and Wang M. Application of Hairy Root Culture for Bioactive Compounds Production in Medicinal Plants. *Curr. Pharm. Biotechnol*. 2021; 22(5): 592-608. doi: 10.2174/ 1389201021666200516155146.

4. Sevón N and Oksman-Caldentey KM. *Agrobacterium rhizogenes*-mediated transformation: root cultures as a source of alkaloids. *Planta Med*. 2002; 68(10): 859-68. doi: 10.1055/s-2002-34924.

5. Chang SS, Park SK, Kim BC, Kang BJ, Kim DU and Nam HG. Stable genetic transformation of *Arabidobsis thaliana* by *Agrobacterium* inoculation *in planta*. *The Plant J.* 1994; 5(4): 551-558. doi: 10.1046/j.1365-313X.1994. 05040551.x.

6. Guillonl S, Jocelyne TG, Pati Kumar PK, Rideau M and Gantet. Hairy root research: recent scenario and exciting prospects. *Curr. Opin. Plant Biol.* 2006; 9(3): 341-346. doi: 10.1016/j.pbi.2006.03.008.

7. Torregrosa L and Bouquet A. *Agrobacterium rhizogenes* and *A. tumefacience* co-transformation to obtain grapevine hairy roots producing the coat protein of grapevine chrome mosaic nepovirus. *PCTOC.* 1997; 49: 53-63. doi: 10. 1023/A:1005854212592.

8. Rao SR and Ravishankar GA. Plant cell cultures: chemical factories of secondary metabolites. *Biotechnol. Adv*. 2002; 20(2): 101-153. doi: 10.1016/s0734-9750(02)00007-1.

9. Thiruvengadam M, Praveen N, John KMM, Yang YS, Kim SH and Chung IM., Establishment of *Momordica charantia* hairy root cultures for the production of phenolic compounds and determination of their biological activities. *PCTOC*. 2014; 118(3): 545-557. doi: 10.1007/s11240-014-0506-4.

10. Huang P, Xia L, Liu W, Jiang R, Liu X, Tang Q, Xu M, Yu L, Tang Z and Zeng J. Hairy root induction and benzylisoquinoline alkaloid production in *Macleaya cordata*. *Sci. Rep.* 2018; 8: 11986. doi: 10.1038/s41598-018-30560-0.

11. Häkkinen ST, Raven N, Henquet M, Laukkanen ML, Anderlei T, Pitkänen JP, Twyman RM, Bosch D, Oksman-Caldentey KM, Schillberg S and Ritala A. Molecular farming in tobacco hairy roots by triggering the secretion of a pharmaceutical antibody. *Biotechnol. Bioeng.* 2013; 111(2): 336-346. doi: 10.1002/bit.25113.

12. Ele Ekouna JP, Boitel-Conti M, Lerouge P, Bardor M and Guerineau F. Enhanced production of recombinant human gastric lipase in turnip hairy roots. *PCTOC*. 2017; 131: 601-610. doi: 10.1007/s11240-017-1309-1.

13. Gutierrez-Valdes N, Häkkinen ST, Lemasson C, Guillet M, Oksman-Caldentey KM, Ritala A and Cardon F. Hairy root cultures-a versatile tool with multiple applications. *Front. Plant Sci.* 2020; 11: 33. doi: 10.3389/fpls. 2020.00033.

14. Murashige T and Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant.* 1962; 15(3): 473-497. doi: 10.1111/j.1399-3054.1962. tb08052.x.

15. Gamborg OL, Miller RA, Ojima K. Nutrient requirements of suspension cultures of soyabean root cells. *Exp. Cell Res.* 1968; 50(1): 151-158.

16. Spurina V, Sovova M, Jirmanova E and Sustackova A. Chromosomal characteristics and

occurrence of main alkaloids in *Datura stramonium* and *Datura wrightii*. *Planta Med.* 1981; 41(4): 366-373.

17. Bonhomme V, Laurain-Mattar D, Lacoux J, Fliniaux MA and Jacquin-Dubreuil A. Tropane alkaloid production by hairy roots of *Atropa belladonna* obtained after transformation with *Agrobacterium rhizogenes* 15834 and *Agrobacterium tumefaciens* containing *rol*A, B, C genes only. *J. Biotech*. 2000; 81(2-3): 151-158. doi: 10.1016/s0168-1656(00)00287-x.

18. Veena V and Taylor CG. *Agrobacterium rhizogenes*: recent developments and promising applications. *In Vitro Cell Dev. Bio. Plant* 2007; 43: 383-403. doi: 10.1007/s11627-007-9096-8

19. Farsi M and Zolala J. Plant Biotechnology. ACECR - Mashhad Branch Publication. 2003; pp: 87-120. [In Persian]

20. Farsi M, Moshtaghi N, Shahriari Ahmadi F, Gordan H and Raisi M. Investigating growth stability and alkaloid content of transgenic hairy roots in *Datura* plant. *Agric. Sci. Ind.* 2005; 19(2): 47-56. [In Persian]

21. Dehghan E, Häkkinen ST, Oksman-Caldentey KM and Shahriari Ahmadi F. Production of tropane alkaloids in diploid and tetraploid plants and in vitro hairy root cultures of Egyptian henbane (*Hyoscyamus muticus* L.). *PCTOC*. 2012; 110: 35-44. doi: 10.1007/s11240-012-0127-8.

22. Georgiev MI, Pavlov AI and Bley T. Hairy root type plant *in vitro* systems as sources of bioactive substances. *Appl. Microbiol. Biotechnol.* 2007; 74: 1175-1185. doi: 10.1007/s00253-007-0856-5.

23. Sathasivam R, Choi M, Radhakrishnan R, Kwon H, Yoon J, Yang SH, Kim JK, Chung YS and Park SU. Effects of various *Agrobacterium rhizogenes* strains on hairy root induction and analyses of primary and secondary metabolites in *Ocimum basilicum*. *Front. Plant Sci.* 2022; 13: 983776. doi: 10.3389/fpls.2022.983776.

24. Thwe A, Arasu MV, Li X, Park CH, Kim SJ, Al-Dhabi NA and Park SU. Effect of different *Agrobacterium rhizogenes* strains on hairy root induction and phenylpropanoid biosynthesis in tartary buckwheat (*Fagopyrum tataricum* gaertn). *Front. Microbiol*. 2016; 7: 318. doi: 10.3389/fmicb.2016.00318.

25. Hamidi Shirghan E, Moshtaghi N, Malekzadeh-Shafaroudi S, Bagheri A and Sharifi A. Effect of the type and concentration of *Agrobacterium rhizogenes* bacteria on the percentage of hairy root emergence in Periwinkle plant. 8th Biotechnology Conference and 4th National Biosafety Conference. 2013.

26. Fan Y, Xu F, Zhou H, Liu X, Yang X, Weng K, Sun X and Lyu S. A fast, simple, high efficient and one-step generation of composite cucumber plants with transgenic roots by *Agrobacterium rhizogenes*-mediated transformation. *PCTOC*. 2020; 141: 207-216. doi: 10.1007/s11240-020-01781-x.

27. Palazon J, Navarro-Ocana A, Hernandez-Vazquez L and Mirjalali MH. Application of metabolic engineering to the production of Scopolamine. *Molecules* 2008; 13(8): 1722-1742. doi: 10.3390/molecules13081722.

28. Silva-Navas J, Moreno-Risueno MA, Manzano C, Pallero-Baena M, Navarro- Neila S, Téllez-Robledo B, Garcia-Mina JM, Baigorri R, Gallego FJ and del Pozo JC. D-Root: a system for cultivating plants with the roots in darkness or under different light conditions. *The Plant J.* 2015; 84(1): 244-255. doi: 10.1111/tpj.12998.

29. Costigan SE, Warnasooriya SN, Humphries BA and Montgomery BL. Root-localized phytochrome chromophore synthesis is required for photoregulation of root elongation and impacts root sensitivity to jasmonic acid in

Arabidopsis. *Plant Physiol.* 2011; 157(3): 1138-1150. doi: 10.1104/pp.111.184689.

30. Correll MJ and Kiss JZ. The roles of phytochromes in elongation and gravitropism of roots. *Plant Cell Physiol.* 2005; 46(2): 317-323. doi: 10.1093/pcp/pci038.

31. Liu CZ, Wang YC, Zhao B, Guo C, Ouyang F, Ye HC and Li GF. Development of a nutrient mist bioreactor for growth of hairy roots. *In Vitro Cell. Dev. Biol.* 1999; 35(3): 271-274. [https://www.jstor.org/stable/4293243].

32. Moyano E, Fornale S, Palazon J, Cusido RM, Bagni N and Pinol MT. Alkaloid production in *duboisia* hybrid hairy root cultures over-expressing the *pmt* gene. *Phytochem.* 2002; 59(7): 697-700. doi: 10.1016/S0031-9422(02)00044-4.

33. Sevon N, Biondi S, Bagni N and Oksman – Caldentey KM. Transgenic *Hyoscyamus muticus.* In: Biotechnology in Agriculture and Forestry 48 (Ed. By Y.P.S Bajaj). Berlin-Heidelberg: Springer velag; 2001: 171-200.

34. Roy A. Hairy root culture an alternative for bioactive compound production from medicinal plants. *Curr. Pharm. Biotechnol*. 2021; 22(1), 136-149. doi: 10.2174/ 1389201021666201229110625.

35. Traverse KKF, Mortensen S, Trautman JG, Danison H, Rizvi NF and Lee-Parsons CWT. Generation of stable *Catharanthus roseus* hairy root lines with *Agrobacterium rhizogenes*. *Methods Mol. Biol*. 2022; 2469: 129-144. doi: 10.1007/978-1-0716-2185-1_11.

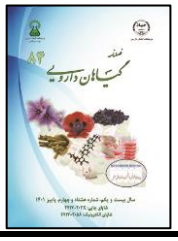
36. Naderian P, Moshtaghi N, Bagheri A, Malekzade Shafaroudi S. Variations in morphological, biochemical and phytochemical traits of diploid and induced tetraploid plants of downy thorn-apple (*Datura innoxia* Mill.). *J. Med. Plants* 2022; 21 (82) 66-79. doi: 10.52547/jmp.21.82.66.

How to cite this article: Naderian P, Moshtaghi N, Bagheri AR, Malekzadeh-Shafaroudi S. Influence of different *Agrobacterium rhizogenes* strains on hairy roots induction and secondary metabolites production in *Datura innoxia* Mill. ***Journal of Medicinal Plants*** 2022; 21(84): 50-64. doi: 10.52547/jmp.21.84.50

فصلنامه گیاهان دارویی

Journal homepage: www.jmp.ir

پژوهشکده گیاهان دارویی
جهاد دانشگاهی



مقاله تحقیقاتی

تأثیر سویه‌های مختلف آگروباکتریوم ریزوژنز بر القای ریشه‌های مویین و تولید متابولیت‌های ثانویه در داتوره تماشایی

پریسا نادریان[۱]، نسرین مشتاقی[۲،*]، عبدالرضا باقری[۲]، سعید ملک‌زاده شفارودی[۲]

[۱] *کارشناس ارشد بیوتکنولوژی کشاورزی، دانشگاه فردوسی مشهد، مشهد، ایران*

[۲] *گروه بیوتکنولوژی و به‌نژادی گیاهی، دانشگاه فردوسی مشهد، مشهد، ایران*

اطلاعات مقاله

گل‌واژگان:
آگروباکتریوم ریزوژنز
کشت
ریشه مویین
شرایط برون‌تنی
هیوسیامین

چکیده

مقدمه: داتوره تماشایی یکی از گیاهان دارویی است که می‌تواند آلکالوئیدهای تروپانی مانند هیوسیامین و اسکوپولامین با خاصیت ضد درد و ضد آسم تولید کند. **هدف**: در این تحقیق اثر عوامل مختلفی از جمله محیط کشت و سویه باکتری بر القای ریشه‌های مویین و تولید متابولیت‌های ثانویه در داتوره تماشایی مورد بررسی قرار گرفت. **روش بررسی**: سویه‌های A4، A13، R1000، MSU، ۱۵۸۳۴، ۲۶۵۶ و ۱۱۳۲۵ از آگروباکتریوم ریزوژنز با دو غلظت مختلف (OD = 0.5, 1) برای همکشتی ریزنمونه‌های برگ و ساقه تهیه شده از گیاهچه‌های ۳-۱ ماهه و ۷-۵ ماهه برای بهینه‌سازی تولید ریشه‌های مویین استفاده شد و سپس رشد ریشه‌ها در شش محیط مختلف بررسی شد. **نتایج**: نتایج تجزیه و تحلیل داده‌ها نشان داد که سویه A4 با تولید ۸۶/۶ درصد ریشه مویین، سویه بهتری نسبت به سایرین بود. همچنین شرایط تاریکی، ریزنمونه‌های جوانتر و غلظت بالاتر باکتری‌ها باعث بیشترین میزان القای ریشه‌های مویین شد. محیط کشت MS ½ به عنوان بهترین محیط برای رشد ریشه‌های مویین شناخته شد. نتایج آنالیز کروماتوگرافی مایع با کارآیی بالا نیز نشان داد که سطح هیوسیامین در ریشه‌های مویین کمتر از ریشه‌های معمولی بود که البته با بیشتر بودن رشد ریشه‌های مویین قابل جبران است. **نتیجه‌گیری**: ظهور و رشد ریشه‌های مویین به عوامل مختلفی از جمله سویه باکتریایی، محیط کشت، ریزنمونه و سن آن و عوامل دیگر بستگی دارد که لازم است در ابتدای هر تحقیقی بهینه شود.

مخففها: MS Medium، محیط کشت موراشیگ و اسکوگ؛ B5، محیط کشت گامبورگ و همکاران؛ YMB، آبگوشت مخمر مالتوز

* نویسنده مسؤول: moshtaghi@um.ac.ir

تاریخ دریافت: ۶ شهریور ۱۴۰۱؛ **تاریخ دریافت اصلاحات:** ۱ آبان ۱۴۰۱؛ **تاریخ پذیرش:** ۴ آبان ۱۴۰۱

doi: 10.52547/jmp.21.84.50