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CALLUS INDUCTION FROM INVIVO EXPLANTS (COTYLEDONARY NODE, LEAF AND SHOOT TIP) OF PEGANUM HARMALA

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ABSTRACT

Peganum harmala is medicinally rich herbaceous plant due to presence of valuable metabolites such as; harmine, harmaline, harmalol etc. This ethnomedicinal plant is at the edge of extinction in Shekhawati region of Rajasthan. For the purpose of conservation of this precious plant micropropagation technique has been applied. It was estimated from the study that wildly grown peganum harmala i.e., invivo explants (cotyledonary nodes, leaves and shoot tips) were capable to induce callus at the concentration of 0.5 to 2.0 mg/l NAA and 0.5 to 2.5 mg/l BAP. Best callus induction obtained were; 1, 1.5 mg/l(NAA, BAP) for cotyledonary nodes, 0.5, 1 mg/l (NAA, BAP) for leaves and 2, 2.5 mg/l(NAA, BAP) for shoot tips. Indirect shoot induction was observed only in invivo cotyledonary nodes at the concentration of 0.5, 1.5(NAA, BAP) and 1, 1.5(NAA, BAP). Leaves and shoot tips were unresponsive for indirect shoot induction. All three invivo explants were unresponsive for direct shoot induction too.

Keywords: Invivo explants, cotyledonary node, leaf, shoot tip, callus, direct shoot induction

INTRODUCTION

Peganum harmala is ethnomedicinal herbaceous plant, belongs to family Nitrariaceae.(Mutasher and Attiya 2019) This valuable plant is distributed to arid and semi regions of North Africa, Middle East, Mediterranean, India, Iran and Pakistan.(Ehsanpour and Sa-Adat 2002) In Rajasthan (India) Peganum harmala found in parts of Hanumangarh, Ganganagar, Nagour and some regions of Shekhawati (Jhunjhunu). Currently this precious medicinally rich plant is at the edge of extinction in Shekhawati region. (Chaudhary 2014) Peganum harmala usually grow up to height 1-2 ft, leaves are narrow and flowers are yellowish white in colour, consisting five petaled structure. (Niroumand, Farzaei, and Amin 2015) Roots are deep and thick (3481,99±12,15µm), which provide resistance against external influencers.(Seilkhan et al. 2019) This wild perennial herb blossom between March to August. Various parts of Peganum harmala including, seeds, bark, roots and fruits have been used as folk medicine (in cardiovascular, gastrointestinal, nervous etc) for a long time in Iran and other countries.(Moloudizargari et al. 2013) In ancient times, seeds of this plant were used by kings in the form of smoke as an antidepressant agent. In a research, it was found that Peganum harmala have antidepressant properties due to presence of harmine; hydrochloride 5.(Doskaliyev et al. 2021) Tribal communities of Rajasthan used this herb as anti-lice agent by mixing its roots powder with mustard oil and apply on scalp.(Kapoor and Kumar 2013) Inhalation of smoke of Peganum harmala relieves toothache and asthma.(Kapoor and Kumar 2013)

In Shekhawati (study area), this valuable plant is almost extinct. So via micropropagation techniques, this precious plant can be re-established. Tissue culture techniques are magical

achievement in scientific world. Although many plants are re-establishing and modifying through micropropagation techniques but very least work has been done on Peganum harmala. So a comparative study or attempts for direct and indirect regeneration was made to propagate Peganum harmala via using invivo (wildly grown) explants including, cotyledonary node, leaf and shoot tip.

MATERIAL AND METHODS

Media preparation

1. For direct organogenesis/ direct shoot induction

Murashige and Skoog nutrient medium (MS medium) is a boon to plant tissue culture techniques. MS medium with addition of sucrose (0.03g/ml) was prepared and pH was adjusted to 5.8±2. Solidifying agent (agar) and hormones for direct shoot induction (without callus formation) i.e., low auxin and high cytokinin (mg/l) were mixed in the medium. Prepared medium was autoclaved at 121^0 C temperatures, 15 psi for 15 minutes.

2. For indirect organogenesis/ callus formation

MS medium with addition of sucrose (0.03g/ml) was prepared and pH was adjusted to 5.8±2. Solidifying agent (agar) and hormones for callus i.e., intermediate amount of auxin and cytokinin (mg/l) were mixed in the medium. Prepared medium was autoclaved at 121^0 C temperatures, 15 psi for 15 minutes.

Explants preparation

Explants (cotyledonary node, leaf and shoop tip) of Peganum harmala were wildly collected (invivo) from study area (Shekhawati) and excised in 5-10 mm. These invivo explants were washed in tap water to remove attached soil and dust particles and further brought to aseptic conditions (laminar air flow) for surface sterilization. Firstly explants were treated with teepol solution; to remove remaining dust particles on the surface of explants. (for 1 min) then washed with autoclaved distilled water (for 2- times). Secondly; explants were immersed in 10% NaOCl solution (commercial bleach) for 2-3 minutes and again washed with autoclaved distilled water. [Soft tissues are sensitive to such chemicals as compare to seeds so chemical application for these tissues should not be for long time.] Surface sterilized seeds were placed over sterilized whatman filter paper to soak excess water from explants.

Direct shoot induction without callus formation

Excised explants (cotyledonary nodes, leaves and shoot tips) were aseptically inoculated into prepared MS medium containing shooting hormones (low auxin and high cytokinin ; table 1) and kept in culture chamber at 25^0 C ±2 temperature, 16 hrs of photoperiod light.

Indirect (callus formation) plant regeneration

Excised explants (cotyledonary nodes, leaves and shoot tips) were aseptically inoculated into prepared MS medium containing callus forming hormones (intermediate amount of auxin and cytokinin ; table 2) and kept in culture chamber at 25^0 C ±2 temperature, 16 hrs of photoperiod light.

Indirect shoot induction

Induced callus from explants; cotyledonary nodes, leaves and shoot tips were sub cultured into MS medium containing shooting hormones (table ; 3). Preparation was kept in culture chamber at 25^0 C ±2 temperature, 16 hrs of photoperiod light.

Table :1 Concentration of plant growth regulators for direct shoot induction (DSI)

Medium no.	Explant	Plant growth regulators (mg/l)	
		NAA	BAP
1.	CN	0.5	1
2.	CN	0.5	1.5
3.	CN	0.5	2
4.	CN	0.5	2.5
5.	CN	0.5	3
6.	L	0.5	1
7.	L	0.5	1.5
8.	L	0.5	2
9.	L	0.5	2.5
10.	L	0.5	3
11.	ST	0.5	1
12.	ST	0.5	1.5
13.	ST	0.5	2
14.	ST	0.5	2.5
15.	ST	0.5	3

CN (Cotyledonary node), L (Leaf), ST (Shoot tip), NAA (α-Naphthalene acetic acid), BAP (Benzyl amino purine)

Table: 2 Concentration of plant growth regulators for callus induction

Medium no.	Explant	Plant growth regulators (mg/l)	
		NAA	BAP
1.	CN	0.5	0.5
2.	CN	0.5	1
3.	CN	1	1
4.	CN	1	1.5
5.	CN	2	2.5
6.	L	0.5	0.5
7.	L	0.5	1
8.	L	1	1
9.	L	1	1.5
10.	L	2	2.5
11.	ST	0.5	0.5
12.	ST	0.5	1
13.	ST	1	1
14.	ST	1	1.5
15.	ST	2	2.5

CN (Cotyledonary node), L (Leaf), ST (Shoot tip), NAA (α-Naphthalene acetic acid), BAP (Benzyl amino purine)

Table : 3 Concentration of plant growth regulators for indirect shoot regeneration

Medium no.	Explant	Plant growth regulators (mg/l)	
		NAA	BAP
1.	CN	0.5	1
2.	CN	0.5	1.5
3.	CN	0.5	2
4.	CN	0.5	2.5
5.	CN	0.5	3
6.	CN	1	1.5
7.	CN	1	3
8.	CN	1	3.5
9.	CN	1	4
10.	CN	1	4.5
11.	L	0.5	1
12.	L	0.5	1.5
13.	L	0.5	2
14.	L	0.5	2.5
15.	L	0.5	3
16.	L	1	1.5
17.	L	1	3
18.	L	1	3.5
19.	L	1	4
20.	L	1	4.5
21.	ST	0.5	1
22.	ST	0.5	1.5
23.	ST	0.5	2
24.	ST	0.5	2.5
25.	ST	0.5	3
26.	ST	1	1.5
27.	ST	1	3
28.	ST	1	3.5
29.	ST	1	4
30.	ST	1	4.5

CN (Cotyledonary node), L (Leaf), ST (Shoot tip), NAA (α-Naphthalene acetic acid), BAP (Benzyl amino purine)

RESULT AND DISCUSSIONS

Invivo explants (CN,L and ST) of Peganum harmala were capable to induce callus in appropriate invitro conditions of light and temperature and suitable callus inducing hormones i.e., NAA (auxin) 0.5 mg/l to 2 mg/l and BAP (cytokinin) 0.5 mg/l to 2.5 mg/l. Best callus inducing concentrations were 1 mg/l NAA and 1.5 mg/l BAP for cotyledonary nodes, 0.5 mg/l NAA and 1 mg/l BAP for leaves and 2 mg/l NAA and 2.5 mg/l BAP for shoot tips. Obtained calluses from all three explants were green, hard and compact in nature. It may be because of addition of NAA in medium, where as BAP in medium was responsible to increase biomass growth. Indirect shoot induction was observed only in cotyledonary nodes. Indirect shoot induction was observed only in cotyledonary nodes at the concentration of 0.5 mg/l NAA +1.5 mg/l BAP and 1 mg/l NAA+ 1.5 mg/l BAP. While there was no induction of shoots in calluses of leaves and shoots tips. All three invivo explants (CN, L and ST) were unresponsive for direct shoot induction. There was only change in colouration (white) obtained in all invivo explants.

It was estimated from the results that, invivo explants were capable for callus induction but further unresponsive to generate shoots and roots at given hormonal concentrations. It may be due to excess or less hormonal concentration/ type of explants (invivo) and other reasons. To solve these mystery further invitro explants can be used for indirect organogenesis in Peganum harmala.

Table: 4 Effect of plant growth regulators for direct shoot induction (DSI)

Medium no.	Invivo explant	Plant growth regulators (mg/l)		Response
		NAA	BAP	
1.	CN	0.5	1	-
2.	CN	0.5	1.5	-
3.	CN	0.5	2	-
4.	CN	0.5	2.5	-
5.	CN	0.5	3	-
6.	L	0.5	1	-
7.	L	0.5	1.5	-
8.	L	0.5	2	-
9.	L	0.5	2.5	-
10.	L	0.5	3	-
11.	ST	.5	1	-
12.	ST	0.5	1.5	-
13.	ST	0.5	2	-
14.	ST	0.5	2.5	-
15.	ST	0.5	3	-

-(No shoot induction; change in colour of explants i.e., green to white)

Table: 5 Effect of plant growth regulators for callus induction

Medium no.	Invivo explant	Plant growth regulators (mg/l)		Response for CI
		NAA	BAP	
1.	CN	0.5	0.5	+
2.	CN	0.5	1	+
3.	CN	1	1	+
4.	CN	1	1.5	++
5.	CN	2	2.5	+
6.	L	0.5	0.5	+
7.	L	0.5	1	++
8.	L	1	1	+
9.	L	1	1.5	+
10.	L	2	2.5	+
11.	ST	0.5	0.5	+
12.	ST	0.5	1	+
13.	ST	1	1	+
14.	ST	1	1.5	+
15.	ST	2	2.5	++

CI (Callus induction), +(formation of callus), ++(best response for callus induction)

Table: 6 Effect of plant growth regulators for indirect shoot regeneration

Medium no.	Explant	Plant growth regulators (mg/l)		Response
		NAA	BAP	
1.	CN	0.5	1	-
2.	CN	0.5	1.5	+
3.	CN	0.5	2	-
4.	CN	0.5	2.5	-
5.	CN	0.5	3	-
6.	CN	1	1.5	+
7.	CN	1	3	-
8.	CN	1	3.5	-
9.	CN	1	4	-
10.	CN	1	4.5	-
11.	L	0.5	1	-
12.	L	0.5	1.5	-
13.	L	0.5	2	-
14.	L	0.5	2.5	-
15.	L	0.5	3	-
16.	L	1	1.5	-
17.	L	1	3	-
18.	L	1	3.5	-
19.	L	1	4	-
20.	L	1	4.5	-
21.	ST	0.5	1	-
22.	ST	0.5	1.5	-
23.	ST	0.5	2	-
24.	ST	0.5	2.5	-
25.	ST	0.5	3	-
26.	ST	1	1.5	-
27.	ST	1	3	-
28.	ST	1	3.5	-
29.	ST	1	4	-
30.	ST	1	4.5	-

+(Shoot induction), -(No response)

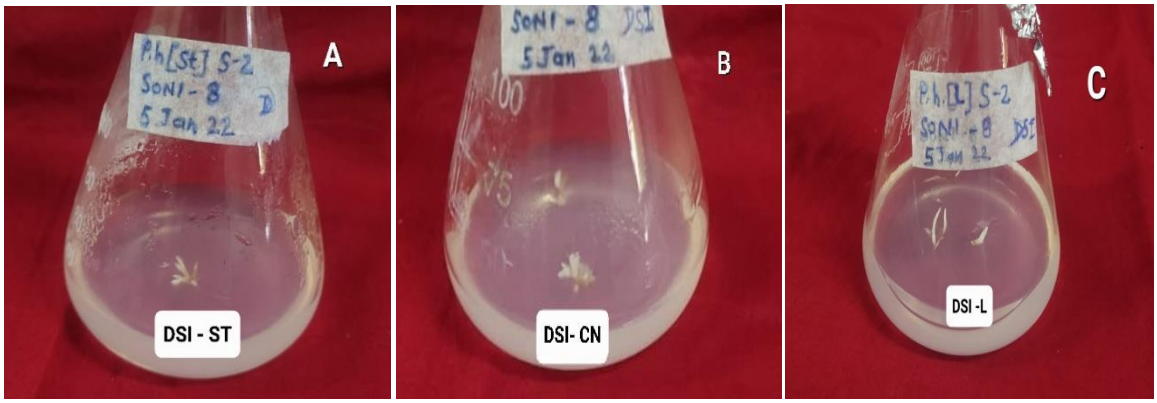

Fig (A, B and C): Unresponsive results of DSI from explants; ST, CN and L of P. harmala

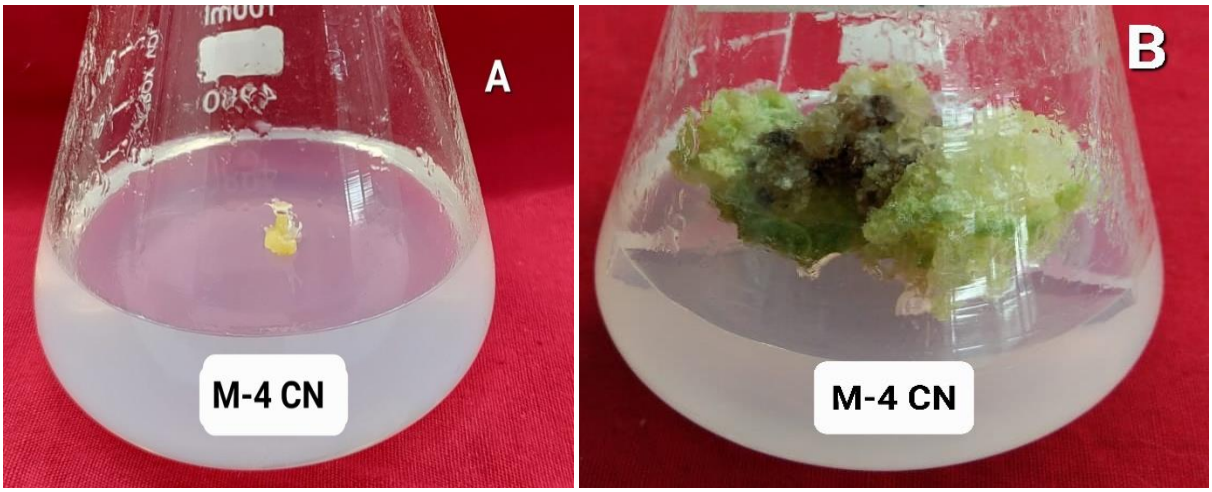

Fig (A and B) : Callus induction (2nd week) and multiplication (8th week) from cotyledonary nodes of P. harmala

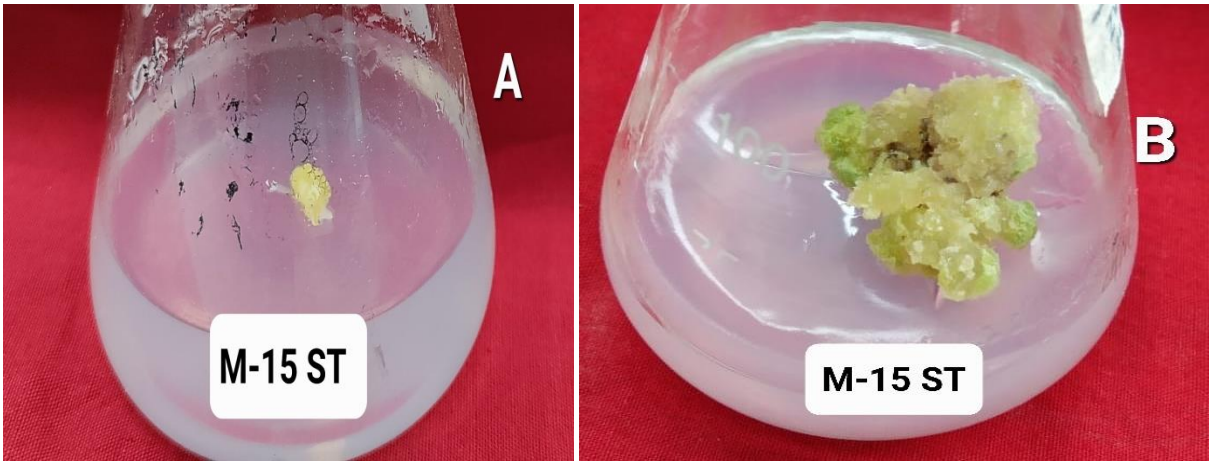

Fig (A and B) : Callus induction (2nd week) and multiplication (8th week) from shoot tips of P. harmala

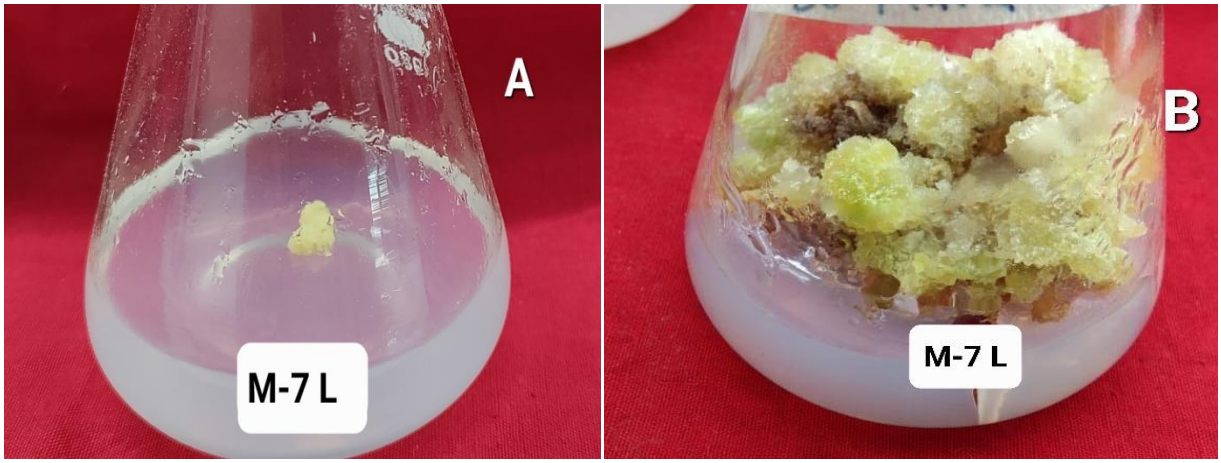

Fig (A and B) Callus induction (2nd week) and multiplication (8th week) from leaves of P. harmala

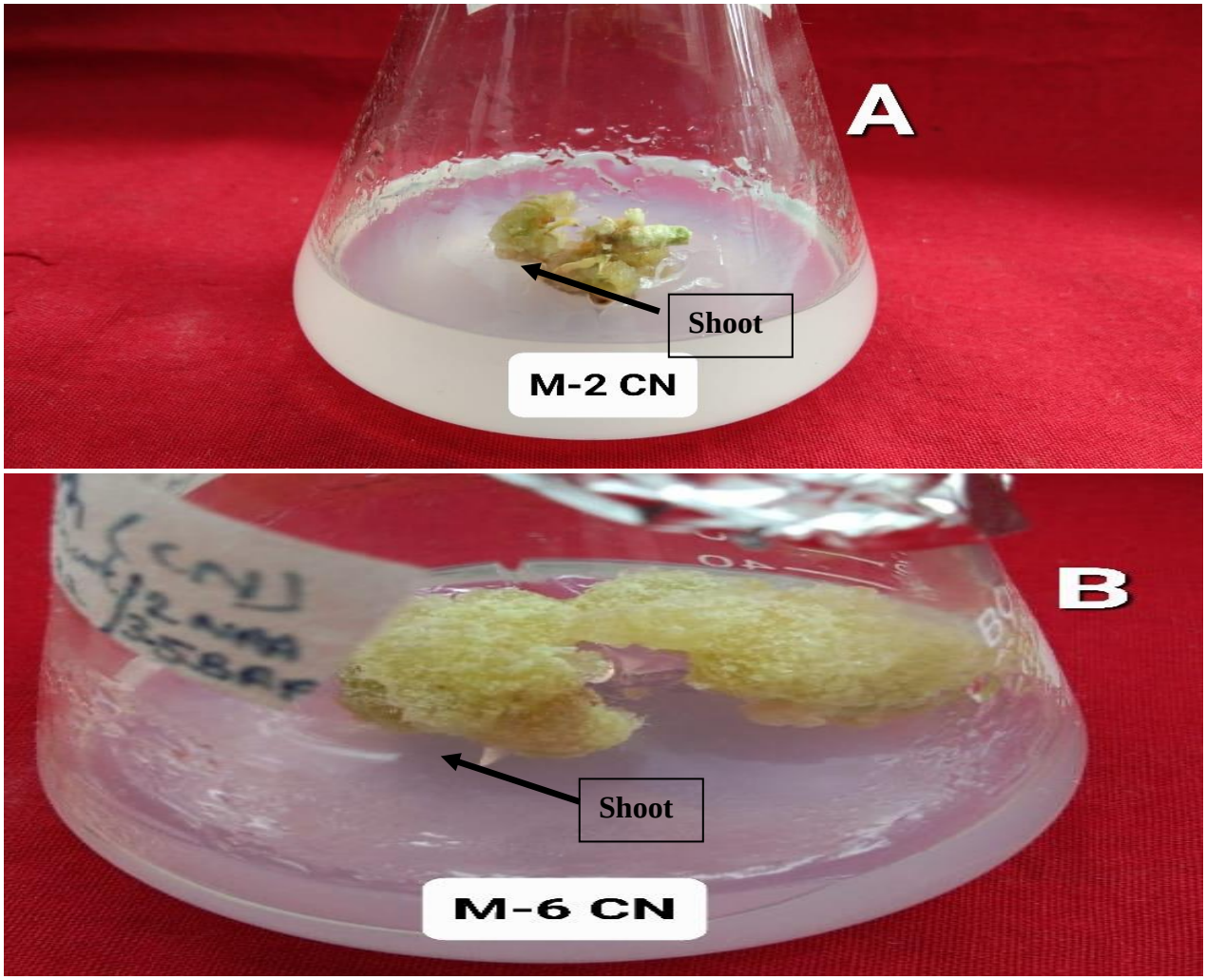

Fig (A and B): Indirect shoot induction from cotyledonary nodes of P. harmala

CONCLUSION

The conclusion of a study or experiment on callus induction from in vitro explants of Peganum harmala might vary based on the specific procedures, conditions, and results obtained in the experiment. However, I can provide a general idea of what conclusions might be drawn based on successful callus induction experiments.

1. **Efficiency of Callus Induction:** The conclusion could discuss the efficiency of callus induction from different explants (cotyledonary node, leaf, shoot tip) of Peganum harmala. This might include a comparison of which explants produced callus more readily or efficiently.
2. **Optimal Conditions for Callus Formation:** Identification of the optimal conditions for callus induction, such as the type and concentration of plant growth regulators (PGRs), culture medium composition, pH, temperature, light conditions, etc. This information is crucial for future studies and applications.
3. **Morphological Characteristics of Callus:** Description of the morphological characteristics of the induced callus, such as color, texture, growth rate, and any observable differences among calli derived from different explants.
4. **Regeneration Potential:** Assessment of the regeneration potential of the induced callus. If the callus showed the ability to regenerate into plantlets or shoots, this would be an important aspect to mention in the conclusion.
5. **Implications for Biotechnological Applications:** Discuss the potential applications of the obtained callus culture in biotechnological aspects, such as secondary metabolite production, genetic transformation, tissue culture-based breeding, or conservation strategies for Peganum harmala.
6. **Further Research Directions:** Highlight any gaps in knowledge or areas that need further investigation. This might include optimizing protocols for enhanced callus induction, understanding molecular mechanisms behind callus formation, or exploring other uses of the callus culture.

REFERENCES

1. Chaudhary, Manju. 2014. "Some Threatened Medicinal Plants of Beer Jhunjhunu Conservation Reserve of Rajasthan , India," no. February: 27–33.
2. Doskaliyev, Aidos, R. B. Seidakhmetova, D. S. Tutai, Kristina Goldaeva, V. K. Surov, and S. M. Adekenov. 2021. "Alkaloids of Peganum Harmala L. and Their Pharmacological Activity." Open Access Macedonian Journal of Medical Sciences 9: 766–75. https://doi.org/10.3889/oamjms.2021.6654.
3. Ehsanpour, Ali Akbar, and Ebrahim Sa-Adat. 2002. "Plant Regeneration from Hypocotyl Culture of Peganum Harmala." Pakistan Journal of Botany 34 (3): 253–56.
4. Kapoor, B. B.S., and Sunil Kumar. 2013. "Ethnomedicinal Plants of Barmer District, Rajasthan Used in Herbal and Folk Remedies." Indian Journal of Pharmaceutical and Biological Research. https://doi.org/10.30750/ijpbr.1.3.11.
5. Moloudizargari, Milad, Peyman Mikaili, Shahin Aghajanshakeri, Mohammad Asghari, and Jalal Shayegh. 2013. "Pharmacological and Therapeutic Effects of Peganum Harmala and Its Main Alkaloids." Pharmacognosy Reviews. https://doi.org/10.4103/0973-7847.120524.
6. Mutasher, H. H., and H. J. Attiya. 2019. "Induced Callus from Seedlings of Peganum Harmala L. And Studying Harmine Compound Concentration in Vitro and in Vivo by GC Analysis." Iraqi Journal of Science 60 (7): 1442–51. https://doi.org/10.24996/ijs.2019.60.7.4.
7. Niroumand, Mina Cheraghi, Mohammad Hosein Farzaei, and Gholamreza Amin. 2015. "Medicinal Properties of Peganum Harmala L. in Traditional Iranian Medicine and Modern Phytotherapy: A Review." Journal of Traditional Chinese Medicine 35 (1): 104–9.

https://doi.org/10.1016/s0254-6272(15)30016-9.

8. Seilkhan, A. S., N. O. Kudrina, N. V. Cherepkova, T. E. Kulmanov, M. S. Kurmanbayeva, Z. A. Inelova, and S. M. Shalgimbayeva. 2019. "Anatomical and Morphological Structure of Peganum Harmala of Almaty Region and Its Therapeutic Properties." Pakistan Journal of Botany 51 (2): 649–55. https://doi.org/10.30848/PJB2019-2(30).