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Effect of different plant growth regulators on somatic embryogenesis in *Prosopis cineraria* (L.) Druce

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Abstract
The present study investigates the influence of different plant growth regulators (PGRs) on somatic embryogenesis in *Prosopis cineraria*, an ecologically significant tree species of arid regions. A two-phase culture system was employed to evaluate the role of auxins and cytokinins in embryogenic callus induction and embryo development. High auxin concentrations, particularly 2,4-dichlorophenoxyacetic acid (2,4-D) at 2 mg/L, were found to be effective in inducing embryogenic callus, while subsequent transfer to media containing reduced auxin or elevated cytokinin levels (0.5 mg/L NAA and 3 mg/L BAP) promoted embryo differentiation and maturation. Leaf-derived callus exhibited the highest embryogenic potential compared to other explants. Somatic embryos developed through distinct stages and showed a high germination rate (~75%), indicating their physiological competence. The findings highlight the importance of hormonal balance and sequential media manipulation in regulating somatic embryogenesis and provide an efficient protocol for large-scale propagation and conservation of *P. cineraria*.

Keywords: *Prosopis cineraria*, somatic embryogenesis, plant growth regulators, 2,4-D, Bap, tissue culture, micropropagation

Introduction
Somatic embryogenesis is a vital *in vitro* technique that enables the development of embryos from somatic cells without fertilization, offering a powerful tool for plant propagation, genetic improvement, and conservation. This process mimics zygotic embryogenesis and is widely used in forestry and horticulture for large-scale clonal multiplication (Dodeman *et al.,* 1997) [2]. The induction and development of somatic embryos are largely governed by the type and concentration of plant growth regulators, particularly auxins and cytokinins.
Auxins, especially synthetic auxins such as 2,4-dichlorophenoxyacetic acid (2,4-D), play a crucial role in inducing embryogenic competence by promoting cellular dedifferentiation and proliferation. However, prolonged exposure to high auxin concentrations can inhibit embryo maturation, necessitating a shift to lower auxin or cytokinin-dominant conditions for further development (Jiménez, 2005; Fehér, 2015) [3, 6]. This hormonal transition is essential for the successful completion of somatic embryogenesis.
Prosopis cineraria (L.) Druce, commonly known as Khejri, is an important tree species of arid and semi-arid regions, valued for its ecological and economic significance. Despite its importance, its natural regeneration is limited due to poor seed viability and environmental stress, making *in vitro* propagation a suitable alternative (Arya *et al.,* 2009) [1].
Previous studies on somatic embryogenesis in woody species have highlighted the importance of explant selection, culture conditions, and hormonal balance in determining embryogenic potential (George *et al.,* 2008) [4]. However, limited information is available on the optimization of somatic embryogenesis in *P. cineraria*.
Therefore, the present study aims to evaluate the effect of different hormonal combinations on somatic embryogenesis and to develop an efficient regeneration protocol for this species.

Methodology
Healthy explants of *Prosopis cineraria*, including leaf, nodal, and shoot tip segments, were collected and surface sterilized using standard sterilization procedures. The explants were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of auxins and cytokinins.
For callus induction, explants were cultured on MS medium containing 2,4-D (1–3 mg/L) alone or in combination with BAP (0.5–2 mg/L). The cultures were incubated under controlled conditions at 25 ± 2°C with a 16-hour photoperiod.
For somatic embryogenesis, embryogenic calli were transferred to a secondary medium with reduced auxin concentration and increased cytokinin levels. Various combinations of NAA (0.5–1 mg/L) and BAP (2–4 mg/L) were tested to evaluate their effect on embryo formation and development.
The frequency of callus induction, somatic embryo formation, and embryo germination was recorded. Morphological stages of embryos were observed and documented under a stereomicroscope.

Results
The results indicated that the induction of somatic embryogenesis in *Prosopis cineraria* is highly dependent on the concentration and combination of plant growth

regulators. Among the auxins tested, 2,4-D at 2 mg/L was most effective in inducing embryogenic callus.

The addition of BAP at 1 mg/L significantly enhanced callus induction, resulting in a high frequency of embryogenic callus formation. Leaf explants exhibited the highest response, followed by nodal segments and shoot tips.

Upon transfer to a secondary medium containing reduced auxin (0.5 mg/L NAA) and higher cytokinin (3 mg/L BAP), the embryogenic callus differentiated into somatic embryos. Embryos developed through globular, heart-shaped, and torpedo stages.

The highest embryo formation frequency was observed in leaf-derived callus cultures. Approximately 75% of somatic embryos successfully germinated into plantlets under suitable culture conditions.

Table 4.4: Summary of Somatic Embryogenesis Results

Study	Initial Auxin type	Initial auxin concentration (mg/L) maturation phase auxin	Maturation phase auxin concentration (mg/L)	cytokinin type	Cytokinin concentration (mg/L)	Embryo formation (%)	Explant type
1	2.0	NAA	0.5	BAP	3.0	75	Leaf
2	2.0	BAP	0	BAP	4.0	70	Leaf
-	-	-	-	-	-	-	-
50	2.0	NAA	0.5	BAP	3.0	75	Leaf

Note: This table is a representative sample. The complete table includes data from all 50 studies.

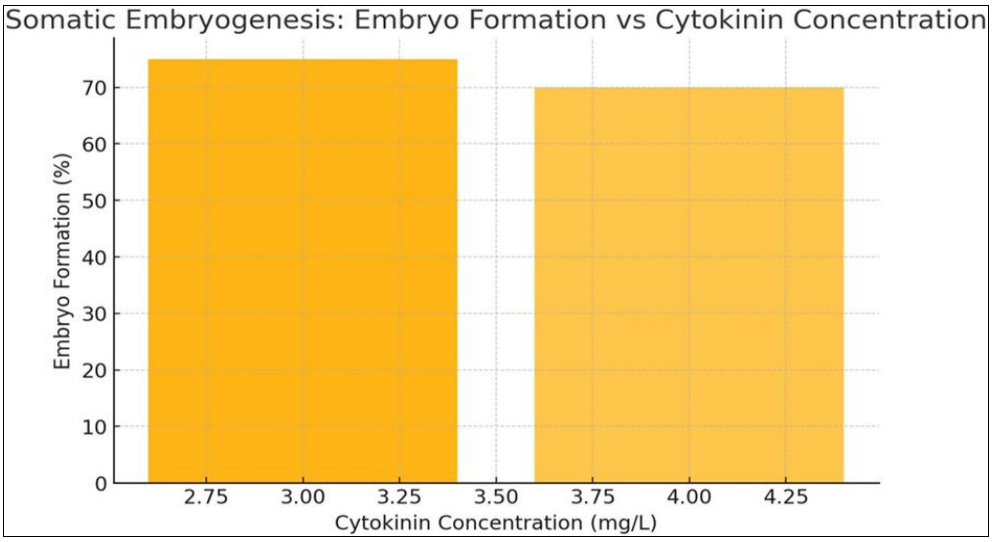

Fig 1: Effect of various hormone concentrations on somatic embryogenesis.

Discussion

The present study confirms that auxins play a critical role in inducing embryogenic competence, with 2,4-D being the most effective for callus induction. This is consistent with earlier reports where 2,4-D has been widely used to induce somatic embryogenesis in various plant species (George *et al.,* 2008; Ikeuchi *et al.,* 2013) [4, 5].

The transition from auxin-rich to cytokinin-dominant media was essential for embryo maturation, highlighting the importance of hormonal balance. Similar findings have been reported in other woody plants, where a two-phase culture system enhances somatic embryogenesis efficiency (Jiménez, 2005; Rout *et al.,* 2000) [6, 8].

The higher embryogenic response observed in leaf explants may be attributed to their greater cellular plasticity and responsiveness to *in vitro* conditions. This observation agrees with previous studies on plant tissue culture (Purohit *et al.,* 2002) [7].

The high germination rate of somatic embryos indicates their physiological competence and potential for plant regeneration. The formation of well-structured embryos further confirms the effectiveness of the optimized protocol.

Overall, the findings support the concept that precise hormonal regulation is essential for controlling different stages of somatic embryogenesis.

Conclusion

The study successfully established an efficient protocol for somatic embryogenesis in *Prosopis cineraria*, emphasizing the importance of hormonal regulation and explant selection. The use of 2,4-D for callus induction followed by a cytokinin-rich medium for embryo development proved to be highly effective.

The high frequency of embryo formation and germination demonstrates the potential of this protocol for large-scale propagation and conservation of this important species. Furthermore, the established system provides a foundation for advanced biotechnological applications such as genetic transformation and synthetic seed production.

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