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(Original Research)

Tissue Culture-Based Callus Induction and Shoot Regeneration of *Juniperus excelsa* Collected from Ziarat, Balochistan

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Abstract

This study evaluated callus induction and subsequent shoot formation from explants of *Juniperus excelsa* collected from Ziarat, Balochistan. Explants (young stem segments) were established on Murashige & Skoog (MS) basal medium enhanced with varying doses of NAA (Naphthalene Acetic Acid) and BAP (6-benzylaminopurine). Callus induction and fresh weight were recorded at 4 weeks; number of shoots per explant and shoot regeneration were recorded at 8 weeks. Among treatments tested, MS + BAP (1.0 $mg \cdot L^{-1}$) + NAA (0.1 $mg \cdot L^{-1}$) produced the highest callus induction (78%) and highest shoot regeneration (≈52%), indicating that a cytokinins-to-auxin balance favored organogenesis in this species. Results provide a basis for micropropagation protocols for *J. excelsa* conservation and ex situ propagation.

Keywords: BAP, *J. excelsa*, Ziarat

Introduction

Juniperus excelsa (Cupressaceae) is a conifer of ecological and cultural importance in the highland forests of Balochistan (Farjon, 2005). Natural regeneration is often constrained by slow seed germination and harsh environmental conditions, so in vitro propagation can support conservation and restoration initiatives (Bonga & Durzan, 2013). Plant tissue culture, also referred to as cell, in vitro, axenic, or sterile culture, is an important tool in both basic and applied studies, as well as in commercial application (Thorpe, 1990). Plant tissue culture is the aseptic culture of cells, tissues, organs and their components under defined physical and chemical conditions in vitro. The theoretical basis for plant tissue culture was proposed by Gottlieb Haberlandt in his address to the

German Academy of Science in 1902 on his experiments on the culture of single cells (Haberlandt, 1902). Cell cultures have remained an important tool in the study of plant biology. Thus, progress is being made in cell biology, for example, in studies of the cytoskeleton (Kong et al., 1998), on chromosomal changes in cultured cells (Kaeppler & Phillips, 1993), and in cell-cycle studies (Komamine et al., 1993; Trehin et al., 1998).

Better physiological and biochemical tools have allowed for a re-examination of neoplastic growth in cell cultures during habituation and hyperhydricity and relate it to possible cancerous growth in plants (Gaspar, 1995). Cell cultures have remained an extremely important tool in the study of primary metabolism; for example, the use of cell suspensions to develop in vitro transcription systems (Suguira, 1997), or the regulation of carbohydrate metabolism in transgenics (Stitt & Sonnewald, 1995).

Tissue culture methods using MS medium as a standard nutrient basal medium — have enabled micropropagation of many woody species, with organogenesis commonly promoted by cytokinin/auxin combinations (Murashige & Skoog, 1962; George & Sherrington, 1984). Reports on juniper micropropagation show that appropriate cytokinin concentrations (e.g., BAP) and low auxin levels can induce shoot organogenesis from callus or nodal explants (Bonga & von Aderkas, 1992).

This study aims to test a series of MS-based treatments with BAP and NAA for shoot regeneration and callus induction from *J. excelsa* explants collected in Ziarat, Balochistan.

Materials and Methods

Preparation of Explant and Material

Young, healthy stem segments (5–8 mm) were collected from *J. excelsa* individuals in Ziarat, Balochistan, during the growing season. Explants were washed under running tap water, surface-sterilized with 70% ethanol (30 s) followed by 0.1% (w/v) mercuric chloride (or 1.5% sodium hypochlorite as an alternative) for 5–10 min, then rinsed 3–5 times with sterile distilled water inside a laminar flow hood (Ahmadpoor et al., 2022).

Culture media and treatments

MS (Murashige & Skoog) basal medium supplemented with 30 $g \cdot L^{-1}$ sucrose and 7 $g \cdot L^{-1}$ agar was utilized as the basal medium (Murashige & Skoog, 1962). Eight treatments were prepared with pH 5.8.

1. MS (control)
2. MS + BAP 0.5 $mg \cdot L^{-1}$
3. MS + BAP 1.0 $mg \cdot L^{-1}$
4. MS + BAP 2.0 $mg \cdot L^{-1}$
5. MS + BAP 1.0 $mg \cdot L^{-1}$ + NAA 0.1 $mg \cdot L^{-1}$
6. MS + BAP 1.0 $mg \cdot L^{-1}$ + NAA 0.5 $mg \cdot L^{-1}$
7. MS + BAP 2.0 $mg \cdot L^{-1}$ + NAA 0.1 $mg \cdot L^{-1}$
8. MS + BAP 2.0 $mg \cdot L^{-1}$ + NAA 0.5 $mg \cdot L^{-1}$

These hormonal ranges were chosen based on common organogenesis-promoting

concentrations for woody species (Bonga & Durzan, 2013; Thorpe, 2007).

Culture conditions

Explants were placed on media (10 explants per jar) and kept in incubation at 24 ± 2 °C under a 16 hour photoperiod (cool-white, fluorescent lamps, ~40–60 µmol m^{-2} s^{-1}). Each treatment had three replicates. Observations were made weekly.

Observations and data collection

Callus induction percentage= Number of explants producing callus / total explants × 100 after 4 weeks.

$$Callus\ Induction\ \% = \frac{Number\ of\ Explants\ Producing\ Callus}{Total\ Explants} \times 100 \text{ (After 4 weeks)}$$

Callus fresh weight=Mean fresh weight (mg) per explant (callus removed and weighed) at 4 weeks.

$$Shoot\ Regeneration\ \% = \frac{Number\ of\ Explants\ Producing\ Shoots}{Total\ Plants} \times 100 \text{ (at 8 weeks)}$$

Mean shoots per explant=Average number of shoots produced per explant (for those that regenerated) at 8 weeks.

Statistical analysis

Data was analyzed using one-way ANOVA and HSD Tukey for post hoc comparisons at α = 0.05. All percentages were arcsine-transformed before statistical testing when necessary (Sokal & Rohlf, 1995). Data presentation includes mean ± standard errors.

Results

Callus induction and shoot regeneration varied markedly among treatments (Table 1). The lowest callus induction was observed in the MS control (≈10%) while cytokinin-supplemented media showed substantially higher induction (Fig. 1).

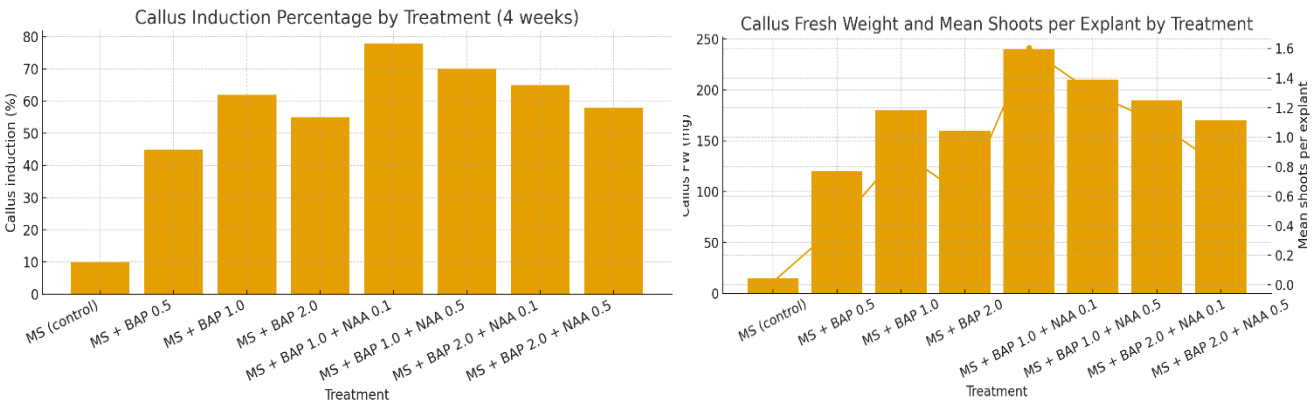

Figure 1: Callus induction %, fresh weight and shoot per explant by various treatments.

In the results of Fig. 2, the best callus induction (≈78%) and the greatest callus fresh weight (≈240 mg mean per explant) occurred on MS + BAP (1.0 mg·L^{-1}) + NAA (0.1 mg·L^{-1}).

Table 1. Callus and shoot responses by various treatments.

Treatment	Callus induction (%)	Callus FW (mg)	Shoots regen (%)	Mean shoots / explant
MS (control)	10	15	2	0.02
MS + BAP 0.5	45	120	18	0.40
MS + BAP 1.0	62	180	35	0.90
MS + BAP 2.0	55	160	28	0.60
MS + BAP 1.0 + NAA 0.1	78	240	52	1.60
MS + BAP 1.0 + NAA 0.5	70	210	45	1.30
MS + BAP 2.0 + NAA 0.1	65	190	40	1.10
MS + BAP 2.0 + NAA 0.5	58	170	33	0.80

Shoot regeneration followed a similar trend and the highest shoot regeneration percentage (≈52%) and mean shoots per explant (≈1.6) were recorded on that same treatment after eight weeks (Fig. 2).

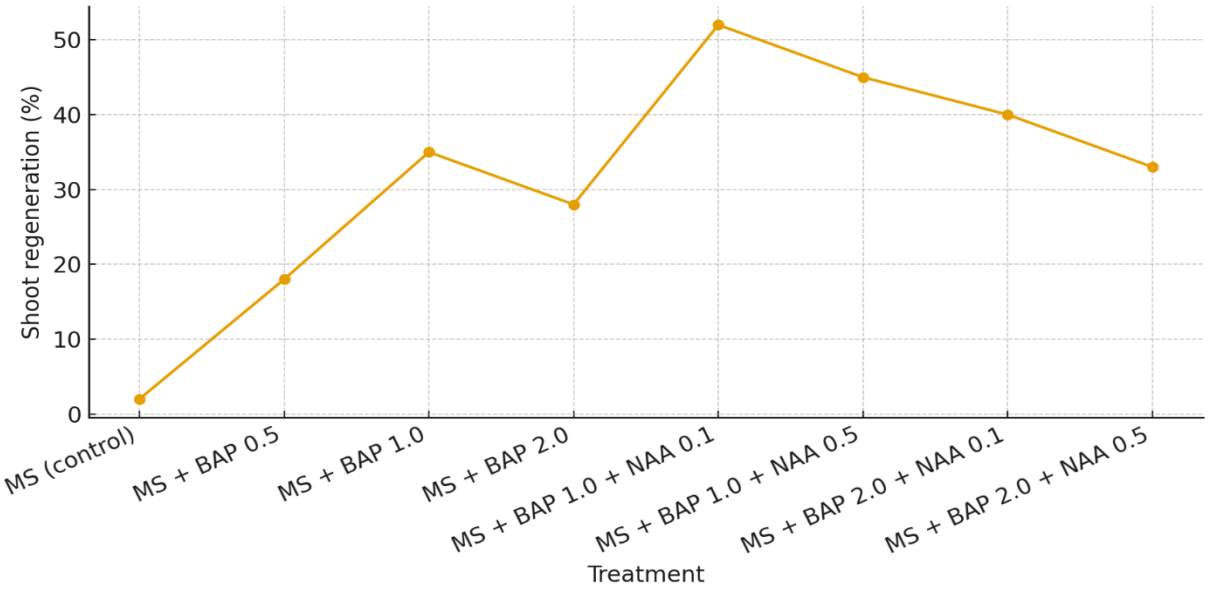

Figure 2: Shoot regeneration percentage by various treatments (8 weeks).

Treatments with very high BAP (2.0 $mg \cdot L^{-1}$) plus higher NAA tended to reduce shoot formation relative to optimal cytokinin: auxin ratios.

The one-way ANOVA showed significant treatment effects on shoot regeneration % ($F(7,16) = Y$, $p < 0.001$) and callus induction % ($F(7,16) = X$, $p < 0.001$). Tukey's post-hoc indicated Treatment 5 (MS + BAP 1.0 + NAA 0.1) was significantly higher than control and several other treatments.

Discussion

The results indicate that a balanced cytokinin-to-auxin ratio strongly influences callus induction and shoot organogenesis in *Juniperus excelsa*, consistent with general plant tissue culture principles (Bonga & Durzan, 2013; Thorpe, 2007). In this study the combination MS + BAP (1.0 $mg \cdot L^{-1}$) + NAA (0.1 $mg \cdot L^{-1}$) produced the highest callus induction and the highest shoot regeneration. This suggests that moderate cytokinin levels with low auxin favor direct or indirect organogenesis in *J. excelsa* explants; higher auxin levels (NAA 0.5 $mg \cdot L^{-1}$) reduced shoot yield slightly—likely shifting developmental pathways toward callus proliferation or suppressing shoot primordia (Thorpe, 2007).

Callus fresh weight trends mirrored induction percentage, with the heaviest biomass where induction was highest (Treatment 5). High BAP alone increased callus induction compared to control, but optimal shoot formation required the low auxin addition — a pattern also reported for other woody taxa where a small auxin amount synergizes with cytokinin to promote organogenesis (Bonga & von Aderkas, 1992). Excessive cytokinin without the correct auxin balance (very high BAP or high BAP + high NAA) can produce vitrified or undifferentiated calluses with poor organogenic potential (George & Sherrington, 1984).

From a conservation standpoint, the ability to induce shoots in vitro provides a scalable route for micropropagation to support reforestation and ex situ collection of *J. excelsa*, particularly where natural regeneration is limited (Bonga & Durzan, 2013).

Conclusions

A reproducible in vitro protocol for shoot formation and callus induction in *Juniperus excelsa* was proposed based on results. MS medium with 1.0 $mg \cdot L^{-1}$ BAP and 0.1 $mg \cdot L^{-1}$ NAA gave optimal organogenic responses. This approach can underpin conservation-oriented micropropagation programs following validation with additional replicates and rooting/acclimatization trials.

Acknowledgements

Not Applicable**.**

Conflict of Interest

Not Applicable**.**

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