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In Vitro Callus Induction in Abrus Precatorius L. Using Different Concentrations and Combinations Of 2,4-D And BAP

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Abstract - The present investigation was undertaken to establish an efficient in vitro callus induction protocol in Abrus precatorius L., an important medicinal plant belonging to the family Fabaceae. The study evaluated the influence of different concentrations of auxin and cytokinin on callus induction using leaf explants. Murashige and Skoog (MS) basal medium was supplemented with different concentrations (1–5 mg/L) of 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BAP), individually and in combination. A total of 15 hormonal treatments were investigated, and each treatment was maintained in triplicate. The cultures were maintained for four weeks under in vitro conditions, and callus growth responses were evaluated. The results revealed considerable variation in callus growth depending on the type and concentration of plant growth regulators. Among the individual 2,4-D treatments, 3 mg/L demonstrated the maximum callus production. The concentrations of 1 and 2 mg/L showed good responses, whereas 4 and 5 mg/L demonstrated better responses. In contrast, individual application of BAP resulted in no callus growth at the tested concentrations. Combined treatments of 2,4-D and BAP at 2 + 2 mg/L and 3 + 3 mg/L showed good callus growth. However, these combinations did not surpass the response obtained with 3 mg/L 2,4-D alone. Callus dry weight was also evaluated as a quantitative parameter of biomass accumulation. The findings indicate that 2,4-D at 3 mg/L is the most promising treatment among the hormonal treatments investigated for callus induction in Abrus precatorius. The study provides a basis for further optimization of in vitro culture conditions and subsequent phytochemical investigations.

Key Words: Abrus precatorius, leaf explant, callus induction, plant tissue culture, MS medium, 2,4-D, BAP, callus proliferation, dry weight.

1.INTRODUCTION

Plant tissue culture is an important biotechnological technique that involves the cultivation of plant cells, tissues and organs under aseptic conditions on a suitable artificial nutrient medium. The technique is based on the principle of cellular totipotency, which refers to the ability of an individual plant cell to regenerate into a complete plant under appropriate nutritional and hormonal conditions.

In recent decades, plant tissue culture has become an essential tool in plant biotechnology, particularly for micropropagation, germplasm conservation, genetic transformation, secondary metabolite production and plant improvement. Among the different tissue culture techniques, callus culture is widely used for studying cellular differentiation, proliferation and physiological responses to plant growth regulators.

A callus is an unorganized mass of actively dividing cells that develops from an explant under suitable in vitro conditions. Callus formation generally involves cellular dedifferentiation followed by continuous cell division. The process is influenced by several factors, including explant type, genotype, nutritional composition, plant growth regulators and environmental conditions.

1.1 Botanical and medicinal importance of Abrus precatorius

Abrus precatorius L. belongs to the family Fabaceae and is commonly known as rosary pea or Indian liquorice. It is a perennial climbing plant characterized by compound leaves, small flowers and distinctive red seeds with a black region near the hilum. The plant has considerable importance in traditional medicinal procedure and contains various phytochemical constituents, including flavonoids, phenolic compounds, alkaloids and other secondary metabolites. The seeds contain abrin, a highly toxic ribosome-inactivating protein. Therefore, appropriate laboratory precautions are necessary while handling plant material, particularly seeds. The establishment of an in vitro culture system provides an opportunity to investigate cellular growth and the production of biologically active compounds under controlled laboratory conditions.

1.2 Importance of callus culture

Callus culture is an important technique for investigating the physiological and biochemical responses of plant tissues under controlled conditions. It is widely employed in plant biotechnology for:

- Studying cellular dedifferentiation and proliferation.
- Developing plant regeneration protocols.
- Producing and investigating secondary metabolites.
- Studying the effects of plant growth regulators.
- Establishing experimental systems for genetic transformation.
- Maintaining plant material under controlled conditions.

The efficiency of callus induction depends largely on the composition of the culture medium and the concentration of plant growth regulators.

1.3 Role of Plant Growth Regulators

Plant growth regulators are essential components of plant tissue culture media as they control cell division, cell expansion, differentiation and morphogenesis. Auxins and cytokinins play a particularly important role in regulating the developmental responses of cultured explants, and their relative concentrations determine the extent of callus induction and proliferation. Therefore, optimization of plant growth regulator concentrations is necessary for achieving efficient callus formation. The present investigation employed two plant growth regulators: 2,4-dichlorophenoxyacetic acid (2,4-D), a

synthetic auxin, and 6-benzylaminopurine (BAP), a synthetic cytokinin.

1.4 Role of 2,4-D in Callus Induction

2,4-D is a synthetic auxin widely used in plant tissue culture for inducing callus formation. It promotes cellular dedifferentiation, stimulates cell division and supports the proliferation of actively growing tissues. It also influences cell expansion and helps maintain undifferentiated cell populations under suitable culture conditions. The effectiveness of 2,4-D depends on its concentration, explant type and physiological condition. An appropriate concentration promotes callus initiation and growth, whereas unsuitable or excessive concentrations may negatively influence cellular development and growth responses.

1.5 Role of BAP in Callus Induction

6-Benzylaminopurine (BAP) is a synthetic cytokinin commonly used in plant tissue culture to regulate cell division, cellular differentiation and shoot development. It influences callus proliferation and interacts with endogenous and externally supplied auxins to regulate the growth and developmental behaviour of cultured tissues. The response to BAP varies depending on its concentration, explant type, species and the availability of auxins in the culture medium. Therefore, its application individually or in combination with auxins helps determine suitable hormonal conditions for callus development and differentiation.

1.6 Interaction Between Auxin and Cytokinin

The interaction between auxins and cytokinins plays a crucial role in regulating plant growth and development under in vitro conditions. The relative balance of these plant growth regulators influences cellular division, callus formation, root initiation and shoot differentiation. Generally, a suitable auxin-to-cytokinin ratio promotes callus induction, although the response varies among plant species and explant types. Therefore, the individual and combined applications of 2,4-D and BAP are important for evaluating their effects and identifying favourable hormonal combinations for efficient callus induction and proliferation in Abrus precatorius L.

1.7 Rationale of the present investigation

The present investigation was designed to evaluate the influence of different concentrations of 2,4-D and BAP on callus induction using leaf explants of Abrus precatorius.

The study involved individual applications of both plant growth regulators at concentrations ranging from 1 to 5 mg/L, together with corresponding equal-concentration combinations.

Particular emphasis was placed on identifying the most effective hormonal treatment for maximum callus production and comparing the responses obtained with auxin alone, cytokinin alone and their combined application.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Healthy and disease-free plants of Abrus precatorius L. were selected as the source of explants. Young and actively growing leaves were carefully collected and transported to the laboratory for establishing in vitro cultures. The plant material was selected to minimize physiological damage and reduce the possibility of microbial contamination during culture establishment.

2.2 Explant Selection

Young leaf explants of Abrus precatorius L. were selected for the present investigation because of their availability and suitability for studying cellular dedifferentiation and callus formation under different hormonal conditions. The selected leaves were carefully cleaned and cut into suitable segments before inoculation onto the prepared culture medium.

2.3 Surface Sterilization

The collected leaf material was initially washed thoroughly under running tap water to remove dust, dirt and other adhering particles. The leaves were then subjected to surface sterilization under aseptic conditions. Initially, the leaf explants were treated with 70% ethanol for 1 minute, followed by treatment with 0.1% mercuric chloride ($HgCl_2$) for 1 minute to eliminate surface-associated microbial contaminants. After sterilization, the explants were washed several times with sterile distilled water (autoclaved distilled water) to remove residual sterilizing agents. Finally, the sterilized leaves were cut into suitable segments using a sterile scalpel and inoculated onto the prepared culture medium under aseptic conditions.

2.4 Preparation of MS Basal Medium

Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) was used for establishing in vitro callus cultures. The medium provided essential macroelements, microelements and vitamins required for plant tissue growth and development. Sucrose was incorporated as a carbon source 3%, while agar 0.8% was used as a solidifying agent. The basal medium was supplemented with different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BAP), either individually or in combination, according to the experimental treatments. The pH of the medium was adjusted to approximately 5.7–5.8 before sterilization. The prepared medium was dispensed into suitable culture vessels and sterilized by autoclaving following standard laboratory procedures.

2.5 Preparation of Hormonal Treatments

Two plant growth regulators, namely 2,4-D and BAP, were used to investigate their individual and combined effects on callus induction in Abrus precatorius L. Both growth regulators were evaluated at five concentrations: 1, 2, 3, 4 and 5 mg/L. The experimental treatments were divided into three groups. The first group consisted of MS medium supplemented individually with 2,4-D at concentrations of 1, 2, 3, 4 and 5 mg/L, while the second group consisted of MS medium supplemented individually with BAP at the same concentrations. The third group included combined applications of 2,4-D and BAP at corresponding concentrations of 1+1, 2+2, 3+3, 4+4 and 5+5 mg/L. Thus, a total of 15 hormonal treatments were evaluated to determine their effects on callus induction and proliferation.

2.6 Experimental Design and Inoculation

The surface-sterilized leaf explants were inoculated onto the respective hormonal treatment media under aseptic conditions. Each hormonal treatment was maintained in three replications to evaluate the consistency of callus development. The inoculated culture vessels were maintained under controlled in vitro conditions for a period of four weeks. During the incubation period, the cultures were regularly observed for callus initiation, proliferation, colour, texture and overall growth response under the different hormonal treatments.

2.7 Incubation Period

The inoculated cultures were maintained for four weeks (28 days) to study the initiation and subsequent development of callus from leaf explants. During this period, observations were recorded regarding the initiation of callus, extent of callus formation, relative growth response, callus colour and texture, and the occurrence of browning or necrosis. The morphological characteristics of the developed callus were also examined to assess the influence of different concentrations and combinations of 2,4-D and BAP on callus development.

2.8 Evaluation of Callus Growth

The effectiveness of different hormonal treatments was evaluated based on the relative amount of callus produced after four weeks of incubation. The growth responses were assessed through visual observations and comparison of callus development among the 15 treatments. Based on the recorded experimental observations, the callus growth responses were classified into three categories: good (*), better (**) and best (***). These categories were used to indicate the relative performance of the different hormonal treatments, with the best rating representing the highest observed callus production.

2.9 Determination of Callus Dry Weight

Callus dry weight was determined as a quantitative parameter for evaluating biomass production under different hormonal treatments. At the end of the four-week incubation period, the developed callus was carefully separated from the explant and culture medium. Excess moisture was removed before subjecting the samples to the established laboratory drying procedure. The callus samples were dried until a constant weight was obtained and subsequently weighed using an analytical balance. Dry-weight measurements were recorded separately for the different hormonal treatments and expressed in grams per experimental unit. These measurements were used to compare the biomass production of callus under individual and combined applications of 2,4-D and BAP.

2.10 Statistical Analysis

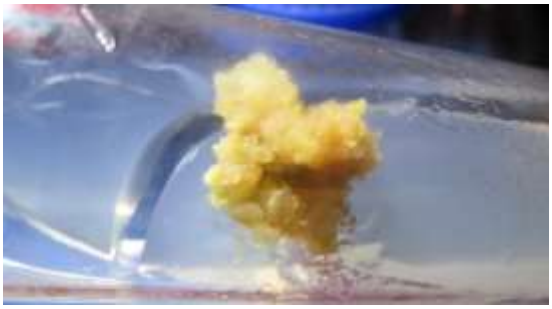
Each hormonal treatment was maintained in three replications to ensure the reliability of the experimental observations. Quantitative data obtained from callus dry-weight measurements may be expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA), followed by an appropriate multiple-comparison test, may be performed to determine the statistical differences among the hormonal treatments. Statistical significance should be reported only after analysing the actual replicate-level measurements.

3. RESULTS

The present investigation evaluated the effect of different concentrations of 2,4-D and BAP on callus induction and proliferation using leaf explants of Abrus precatorius.

The observations were recorded after four weeks of incubation on MS basal medium supplemented with individual and combined hormonal treatments.

Figure 1. Significant callus induction by using 2,4-D 3mg/L



3.1 Effect of different concentrations of 2,4-D on callus induction

The individual application of 2,4-D showed a favourable influence on callus formation in Abrus precatorius. Among the five concentrations investigated, 3 mg/L 2,4-D demonstrated the maximum callus production. The treatments containing 1 and 2 mg/L 2,4-D showed good callus growth, whereas concentrations of 4 and 5 mg/L exhibited better growth responses. The maximum response was recorded at 3 mg/L, indicating that this concentration provided the most favourable conditions for callus proliferation among the tested individual auxin treatments.

Table 1. Effect of different concentrations of 2,4-D on callus growth in Abrus precatorius L.

Treatment	2,4-D (mg/L)	Callus growth response	Dry weight (g) Mean
T1	1	Good (*)	0.4
T2	2	Good (*)	0.5
T3	3	Best (***)	1.2
T4	4	Better (**)	0.6
T5	5	Better (**)	0.6

Interpretation: The maximum callus production was obtained with 3 mg/L 2,4-D. The comparatively favourable response indicates the importance of auxin concentration in regulating cellular dedifferentiation and callus proliferation.

3.2 Effect of different concentrations of BAP on callus induction

BAP was used individually at concentrations of 1, 2, 3, 4 and 5 mg/L to investigate the influence of cytokinin on callus formation.

The observations indicated poor callus growth under the individual BAP treatments. Compared with the individual 2,4-D treatments, BAP alone was less effective in promoting callus proliferation under the investigated experimental conditions.

This response suggests that the selected leaf explants responded more favourably to auxin supplementation than to cytokinin supplementation alone.

Table 2. Effect of different concentrations of BAP on callus growth in Abrus precatorius L.

Treatment	BAP (mg/L)	Callus growth response
T6	1	Nil
T7	2	Nil
T8	3	Nil
T9	4	Nil
T10	5	Nil

Interpretation: Individual application of BAP resulted in poor callus growth at the tested concentrations. This indicates that BAP alone was comparatively less effective than 2,4-D for callus induction in the investigated leaf explants.

3.3 Effect of combined application of 2,4-D and BAP

The combined application of auxin and cytokinin was investigated to evaluate their interaction during callus formation. Five combinations were tested using corresponding concentrations of 2,4-D and BAP. The observations revealed that combinations containing 2 mg/L and 3 mg/L of each plant growth regulator demonstrated good callus growth. However, these combinations did not produce callus growth superior to that obtained with 3 mg/L 2,4-D alone.

Table 3. Effect of combined concentrations of 2,4-D and BAP on callus growth in Abrus precatorius L.

Treatment	2,4-D (mg/L)	BAP (mg/L)	Growth response
T11	1	1	Nil
T12	2	2	Good (*)
T13	3	3	Good (*)
T14	4	4	Nil
T15	5	5	Nil

Interpretation: The combined application of 2,4-D and BAP at 2 + 2 mg/L and 3 + 3 mg/L resulted in good callus growth. However, the combined treatments were comparatively less effective than 3 mg/L 2,4-D alone.

3.4 Comparative evaluation of hormonal treatments

The comparative evaluation of all 15 treatments demonstrated that the response of leaf explants varied according to the type and concentration of plant growth regulators.

Among the individual treatments, 2,4-D was more effective than BAP for promoting callus growth.

The maximum callus production was recorded at 3 mg/L 2,4-D. Although combined treatments at 2 + 2 mg/L and 3 + 3 mg/L showed good growth, their responses were not superior to the best individual auxin treatment.

Table 4. Comparative summary of the hormonal treatments showing favourable callus growth.

Treatment	Hormonal composition	Concentration (mg/L)	Response
T1	2,4-D	1	Good (*)
T2	2,4-D	2	Good (*)
T3	2,4-D	3	Best (***)
T4	2,4-D	4	Better (**)
T5	2,4-D	5	Better (**)
T12	2,4-D + BAP	2 + 2	Good (*)
T13	2,4-D + BAP	3 + 3	Good (*)

The individual BAP treatments exhibited poor growth, while the other combined treatments were not assigned a positive growth rating in the recorded observations.

3.5 Statistical Analysis of Dry Weight of Callus

The dry-weight data were statistically analysed to evaluate the effect of different concentrations and combinations of 2,4-D (2,4-Dichlorophenoxyacetic acid) and BAP (6-Benzylaminopurine) on callus biomass production in Abrus precatorius. The experiment consisted of 15 hormonal treatments, with three replicates per treatment. The statistical analysis included the calculation of mean dry weight, standard deviation, standard error, coefficient of variation and one-way analysis of variance (ANOVA). The treatment means were compared to identify the hormonal composition that supported maximum callus biomass accumulation after four weeks of culture.

Table 5. Dry weight of callus obtained under different hormonal treatments. Dry weight (g) – Three replicates

Treatment	Hormonal treatment	R1 (g)	R2 (g)	R3 (g)	Mean (g)
T1	2,4-D 1 mg/L	0.3	0.4	0.5	0.4
T2	2,4-D 2 mg/L	0.4	0.5	0.6	0.5
T3	2,4-D 3 mg/L	1.0	1.3	1.3	1.2
T4	2,4-D 4 mg/L	0.5	0.6	0.7	0.6
T5	2,4-D 5 mg/L	0.7	0.5	0.6	0.6
T6	BAP 1 mg/L	0	0	0	0
T7	BAP 2 mg/L	0	0	0	0
T8	BAP 3 mg/L	0	0	0	0
T9	BAP 4 mg/L	0	0	0	0
T10	BAP 5 mg/L	0	0	0	0
T11	2,4-D 1 + BAP 1 mg/L	0	0	0	0
T12	2,4-D 2 + BAP 2 mg/L	0.3	0.4	0.5	0.4
T13	2,4-D 3 + BAP 3 mg/L	0.4	0.5	0.6	0.5
T14	2,4-D 4 + BAP 4 mg/L	0.1	0.2	0.3	0.2
T15	2,4-D 5 + BAP 5 mg/L	0	0	0	0

Table 6. Mean dry weight, standard deviation and standard error of callus under different hormonal treatments.

Treatment	Mean (g)	SD	SE
T1	0.40	0.100	0.058
T2	0.50	0.100	0.058
T3	**1.20**	0.173	0.100
T4	0.60	0.100	0.058
T5	0.60	0.100	0.058
T6	0.00	0.000	0.000
T7	0.00	0.000	0.000
T8	0.00	0.000	0.000
T9	0.00	0.000	0.000
T10	0.00	0.000	0.000
T11	0.00	0.000	0.000
T12	0.40	0.100	0.058
T13	0.50	0.100	0.058
T14	0.20	0.100	0.058
T15	0.00	0.000	0.000

Interpretation: The highest mean dry weight (1.20 g) was recorded in T3 containing 3 mg/L 2,4-D. This was followed by T4 (4 mg/L 2,4-D) and T5 (5 mg/L 2,4-D), both showing a mean dry weight of 0.60 g. The BAP-alone treatments (T6–T10) and T11 and T15 (combined hormonal treatments) showed no measurable callus biomass. Among the combined hormonal treatments, T13 (3 mg/L 2,4-D + 3 mg/L BAP) recorded the highest mean dry weight of 0.50 g.

3.6 One-way analysis of variance (ANOVA)

One-way ANOVA was performed to determine whether the differences in mean dry weight among the 15 hormonal treatments were statistically significant.

Table 7. Analysis of variance for dry weight of callus.

Source of variation	df	Sum of squares	Mean square	F-value
Treatments	14	5.188	0.3706	**55.586**
Error	30	0.200	0.0067	
Total	44	5.388		

Statistical results calculated from the 45 individual replicate observations.

ANOVA results:

- Calculated F-value = 55.586
- Degrees of freedom = 14 and 30
- F-critical value at 5% significance = 2.037
- p-value = 1.54×10^{-17}

Since the calculated F-value is considerably higher than the critical F-value (55.586 greater than 2.037), the treatment effect is statistically significant at the 5% level. The p-value is also considerably lower than 0.05, indicating significant differences in dry-weight accumulation among the hormonal treatments.

3.8 Non-parametric statistical confirmation

Since several treatments (T6–T11 and T15) recorded zero dry weight in all three replicates, the data contain multiple zero-variance groups. This creates a concern regarding the equal-variance assumption of conventional ANOVA. Therefore, the Kruskal–Wallis test was additionally performed as a non-parametric confirmation.

Statistical parameter	Result
Kruskal–Wallis H statistic	42.415
Degrees of freedom	14
p-value	0.000106
Significance level	0.05
Result	Significant

The Kruskal–Wallis test also indicated a statistically significant difference among the hormonal treatments (p less than 0.05). Thus, the overall treatment effect is supported by both statistical approaches.

3.8 Biological interpretation of dry-weight accumulation

The dry-weight analysis demonstrated that the concentration of 2,4-D played an important role in callus biomass accumulation in Abrus precatorius. The treatment containing 3 mg/L 2,4-D (T3) produced the maximum dry weight of 1.20 g, indicating that this concentration was most favourable among the tested treatments for callus biomass production. At lower concentrations (1 and 2 mg/L), relatively lower dry-weight accumulation was observed. Increasing the concentration to 4 and 5 mg/L did not further increase biomass production. This suggests that the response to 2,4-D was concentration-dependent, with 3 mg/L providing the highest biomass accumulation under the experimental conditions. BAP alone failed to produce measurable callus biomass at all tested concentrations. Similarly, the combined application of 2,4-D and BAP produced variable responses. The highest biomass among the combined treatments was recorded with 3 mg/L 2,4-D + 3 mg/L BAP (0.50 g).

4. CONCLUSION

The statistical analysis revealed significant differences in callus dry-weight accumulation among the different hormonal treatments. Among all 15 treatments, T3 (3 mg/L 2,4-D) was the most effective treatment, producing the highest mean dry weight of 1.20 g after four weeks of culture. The results indicate that 2,4-D alone, particularly at 3 mg/L, was more effective for callus biomass production than BAP alone or the tested combinations of 2,4-D and BAP.

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