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(RESEARCH ARTICLE)

Optimization of culture conditions for enhanced L-DOPA biotransformation in *Solanum tuberosum* L.

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Abstract

The present study investigates the production of L-3,4-dihydroxyphenylalanine (L-DOPA) using callus and cell suspension cultures of *Solanum tuberosum* L. Leaf explants cultured on half-strength Murashige and Skoog (MS) medium supplemented with plant growth regulators showed efficient callus induction, with the highest response observed in medium containing NAA (2.0 mg L^{-1}), kinetin (2.0 mg L^{-1}), and 2,4-D (1.0 mg L^{-1}). The resulting friable callus was successfully used to establish cell suspension cultures, which exhibited significantly higher tyrosinase activity compared to solid callus cultures.

Biotransformation studies demonstrated that L-DOPA production increased with incubation time, reaching a maximum at 36 hours. Optimization experiments revealed that substrate concentration significantly influenced production, with 5 mM L-tyrosine identified as optimal for achieving a balance between yield and conversion efficiency. Higher substrate concentrations increased total L-DOPA production but reduced conversion efficiency, likely due to enzyme saturation. Additionally, 2,4-D concentration was found to play a critical role in regulating callus growth, enzyme activity, and metabolite production.

Statistical analysis confirmed significant differences among treatments, and a strong positive correlation was observed between tyrosinase activity and L-DOPA production. The study demonstrates that *Solanum tuberosum* cell suspension cultures represent a promising and sustainable system for L-DOPA production. Further work on downstream processing, including isolation and purification, is recommended for potential industrial application.

Keywords: L-DOPA; *Solanum tuberosum*; Callus culture; Cell suspension culture; Tyrosinase; Biotransformation; Secondary metabolites; Substrate optimization

1. Introduction

L-3,4-dihydroxyphenylalanine (L-DOPA) is a clinically important catecholamine precursor widely used in the treatment of Parkinson's disease. Due to its crucial therapeutic role, there is increasing interest in developing efficient and sustainable methods for its production. Conventional chemical synthesis of L-DOPA often involves complex procedures and generates unwanted by-products, making biological production systems more desirable [1].

Plant tissue culture techniques offer a promising alternative for the production of valuable secondary metabolites under controlled conditions. Among various plant species, *Solanum tuberosum* has been explored for its ability to produce phenolic compounds and enzymatic systems involved in biotransformation processes [2]. Callus and cell suspension cultures, in particular, provide a uniform and scalable system for metabolite production, enabling better control over environmental and nutritional factors [3].

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The enzyme tyrosinase (EC 1.14.18.1) plays a key role in the hydroxylation of L-tyrosine to L-DOPA, representing a critical step in melanin biosynthesis pathways [4]. The efficiency of this bioconversion process depends on several factors, including enzyme activity, substrate concentration, culture conditions, and plant growth regulators [5].

Optimization of culture conditions, especially the concentration of auxins and cytokinins, significantly influences callus induction, cell proliferation, and metabolic activity. Synthetic auxins such as 2,4-dichlorophenoxyacetic acid (2,4-D) and α-naphthaleneacetic acid (NAA), along with cytokinins like kinetin, have been widely used to enhance callus growth and secondary metabolite production [6].

Furthermore, substrate concentration plays a crucial role in determining both the yield and efficiency of biotransformation. While increasing substrate levels may enhance product formation, excessive concentrations can lead to enzyme inhibition or reduced conversion efficiency due to metabolic limitations [7].

In this context, the present study aims to:

- Establish callus and cell suspension cultures of *Solanum tuberosum*,
- Evaluate tyrosinase activity in different culture systems,
- Investigate the biotransformation of L-tyrosine to L-DOPA, and
- Optimize key parameters such as substrate concentration and growth regulator levels for enhanced L-DOPA production.

2. Materials and methods

2.1. Plant Material and Explant Preparation

Healthy leaves of *Solanum tuberosum* L. from Thiruvananthapuram were collected and used as explants. The explants were washed thoroughly under running tap water followed by surface sterilization using 70% ethanol for 30 seconds and 0.1% mercuric chloride for 3–5 minutes. Subsequently, the explants were rinsed 4–5 times with sterile distilled water under aseptic conditions [8].

2.2. Callus Induction and Culture Conditions

Sterilized leaf explants were inoculated onto half-strength Murashige and Skoog (MS) medium supplemented with varying concentrations of NAA, kinetin, and 2,4-D as per experimental design. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 15 minutes.

Cultures were incubated at 25 ± 2°C under a 16-hour photoperiod. Callus induction frequency and growth index were recorded after 4 weeks of culture. The growth index (GI) was calculated using the formula:

$$GI = \frac{Final\ fresh\ weight - Initial\ fresh\ weight}{Initial\ fresh\ weight}$$

2.3. Establishment of Cell Suspension Cultures

Friable calli obtained from optimized treatment were transferred to liquid half-strength MS medium containing the same growth regulators. Cultures were maintained in 250 mL Erlenmeyer flasks containing 50 mL medium and incubated on a rotary shaker at 110–120 rpm under controlled temperature conditions.

Suspension cultures were subcultured at 7-day intervals to maintain active growth [9].

2.4. Tyrosinase Activity Assay

Tyrosinase activity was determined in both callus and suspension cultures using L-tyrosine as substrate. The reaction mixture contained phosphate buffer (pH 6.8), substrate solution, and enzyme extract.

The increase in absorbance due to dopachrome formation was measured spectrophotometrically at 475 nm. One unit of enzyme activity was defined as the amount of enzyme required to produce 1 µmol of product per minute under assay conditions [10].

Protein content was estimated using the Lowry method, and specific activity was expressed as U mg^{-1} protein [11].

2.5. Biotransformation of L-Tyrosine to L-DOPA

Cell suspension cultures (10% v/v inoculum) were incubated with L-tyrosine at defined concentrations. The cultures were maintained under shaking conditions, and samples were collected at regular intervals (0–48 hours).

L-DOPA concentration was estimated spectrophotometrically based on standard calibration curves. Residual L-tyrosine concentration was also measured to determine substrate utilization [12].

Conversion efficiency (%) was calculated as:

$$Conversion\ Efficiency = \frac{L-DOPA\ produced}{Initial\ L-tyrosine} \times 100$$

Conversion efficiency=Initial L-tyrosineL-DOPA produced×100

2.6. Optimization Studies

2.6.1. Effect of Substrate Concentration

Different concentrations of L-tyrosine (1–15 mM) were evaluated to determine their effect on L-DOPA production and conversion efficiency.

2.6.2. Effect of 2,4-D Concentration

The influence of 2,4-D (0.5–1.5 mg L^{-1}) on callus growth, tyrosinase activity, and L-DOPA production was assessed under optimized culture conditions.

2.7. Statistical Analysis

All experiments were performed in triplicate, and results are expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way ANOVA followed by Tukey's post hoc test at $p < 0.05$. Student's t-test was used for pairwise comparisons, and Pearson correlation analysis was conducted to evaluate relationships between variables [13].

3. Results

3.1. Callus Induction and Growth Response

Leaf explants of *Solanum tuberosum* cultured on half-strength Murashige and Skoog (MS) medium supplemented with different combinations of plant growth regulators exhibited successful callus induction within 15–20 days of inoculation. Initially, the explants showed slight swelling at the cut ends, followed by the formation of cream-coloured compact callus. Upon subsequent growth, the callus became pale yellow and friable in texture, particularly in media containing higher auxin concentrations.

Among the treatments tested, the medium supplemented with NAA (2.0 mg L^{-1}), Kinetin (2.0 mg L^{-1}), and 2,4-D (1.0 mg L^{-1}) produced the highest callus induction frequency (92.8%) and growth index (3.67 ± 0.21). The induced callus was friable and actively proliferating, making it suitable for the establishment of suspension cultures.

Table 1 Effect of different growth regulator combinations on callus induction from leaf explants of *Solanum tuberosum*

Treatment	NAA (mg L^{-1})	Kinetin (mg L^{-1})	2,4-D (mg L^{-1})	Callus induction (%)	Growth index (GI)	Callus texture
T1	1.0	1.0	0.5	56.3 ± 2.5^{a}	1.42 ± 0.12^{a}	Compact
T2	2.0	2.0	0.5	78.5 ± 3.1^{b}	2.15 ± 0.18^{b}	Friable
T3	2.0	2.0	1.0	92.8 ± 2.4^{c}	3.67 ± 0.21^{c}	Friable
T4	3.0	2.0	1.0	85.2 ± 3.6^{bc}	3.10 ± 0.25^{bc}	Friable

Values represent mean ± SD of three independent experiments with 10 explants per replicate. Values with different superscripts differ significantly at $p < 0.05$.

One-way ANOVA revealed significant differences among treatments in both callus induction frequency and growth index ($p < 0.001$). Treatment T3 was selected for further experiments due to superior biomass accumulation and friable texture.

3.2. Tyrosinase Activity in Callus and Cell Suspension Cultures

Friable calli obtained from the optimized treatment (T3) were transferred to liquid half-strength MS medium for the establishment of cell suspension cultures. The suspension cultures showed active proliferation under continuous shaking conditions and developed a uniform cell population within 7–8 days.

Tyrosinase activity was estimated in both solid callus cultures and cell suspension cultures. Suspension cultures exhibited significantly higher enzyme activity compared to solid callus cultures.

Table 2 Tyrosinase activity in callus and cell suspension cultures of *Solanum tuberosum*

Culture type	Tyrosinase activity (U mL^{-1})	Specific activity (U mg^{-1} protein)
Solid callus	1.12 ± 0.09^{a}	0.34 ± 0.03^{a}
Suspension culture	1.87 ± 0.11^{b}	0.58 ± 0.04^{b}

Values represent mean ± SD (n = 3). Values with different superscripts differ significantly at $p < 0.05$.

Student's t-test demonstrated that suspension cultures possessed significantly greater tyrosinase activity than solid callus cultures ($p < 0.01$). The enhanced activity observed in suspension cultures may be attributed to improved nutrient uptake, aeration, and metabolic activity under liquid culture conditions.

3.3. Biotransformation of L-Tyrosine to L-DOPA

Cell suspension cultures derived from optimized friable calli were utilized for the biotransformation of L-tyrosine into L-DOPA. A 10% (v/v) inoculum was incubated with 5 mM L-tyrosine substrate, and L-DOPA production was monitored over a period of 48 hours.

L-DOPA production increased progressively with incubation time and reached a maximum concentration of 802.1 ± 14.2 mg L^{-1} after 36 hours. However, the highest conversion efficiency (80.2%) was also recorded at this stage. Beyond 36 hours, a slight reduction in L-DOPA concentration was observed, possibly due to oxidation of L-DOPA to dopaquinone or further enzymatic degradation.

Table 3 Time course of L-DOPA production from L-tyrosine by cell suspension cultures

Time (h)	L-DOPA produced (mg L^{-1})	Conversion efficiency (%)	Residual L-tyrosine (mg L^{-1})
0	0.00	0.0	1000.0
6	124.5 ± 5.2^{a}	12.5	865.2 ± 12.1
12	342.8 ± 10.3^{b}	34.3	648.5 ± 15.4
24	785.6 ± 15.7^{c}	78.6	210.3 ± 10.2
36	802.1 ± 14.2^{c}	80.2	198.7 ± 9.8
48	795.4 ± 16.1^{c}	79.5	205.6 ± 11.3

Values represent mean ± SD (n = 3). Values with different superscripts differ significantly at $p < 0.05$.

The gradual decline in residual L-tyrosine concentration confirmed active substrate utilization by the suspension cultures. The results indicate that the biotransformation process was highly efficient during the exponential growth phase of the culture.

4. Optimization of L-DOPA Production

4.1. Effect of Initial L-Tyrosine Concentration

Different concentrations of L-tyrosine were evaluated to determine their influence on L-DOPA production. The results showed that increasing substrate concentration enhanced L-DOPA production up to 10 mM. However, conversion efficiency decreased at higher substrate concentrations.

Although maximum L-DOPA yield (1105.8 ± 28.6 mg L^{-1}) was observed at 15 mM substrate concentration, the highest overall conversion efficiency was obtained at 5 mM. Therefore, 5 mM L-tyrosine was considered optimal for balanced biotransformation performance.

Table 4 Effect of initial substrate concentration on L-DOPA production

L-Tyrosine (mM)	L-DOPA Produced (mg L^{-1})	Conversion Efficiency (%)
1	102.4 ± 3.2[a]	20.5
2	205.8 ± 5.6[b]	41.1
5	512.6 ± 10.8[c]	61.5
10	1018.3 ± 18.7[d]	81.4
15	1525.7 ± 25.9[e]	90.2

Values represent mean ± SD (n = 3). Values with different superscripts differ significantly at $p < 0.05$.

The reduction in conversion efficiency at higher substrate concentrations may be attributed to substrate saturation or partial inhibition of tyrosinase activity.

4.2. Effect of 2,4-D Concentration on L-DOPA Production

The concentration of 2,4-D significantly affected callus growth, tyrosinase activity, and L-DOPA production. Among the concentrations tested, 1.0 mg L^{-1} 2,4-D produced the highest callus growth index and tyrosinase activity, resulting in maximum L-DOPA production.

Table 5 Effect of 2,4-D concentration on callus growth, tyrosinase activity, and L-DOPA production

2,4-D concentration (mg L^{-1})	Callus growth index	Tyrosinase activity (U mL^{-1})	L-DOPA produced (mg L^{-1})
0.5	2.21 ± 0.14[a]	0.98 ± 0.07[a]	412.3 ± 12.1[a]
1.0	3.67 ± 0.21[c]	1.87 ± 0.11[c]	785.6 ± 15.7[c]
1.5	3.42 ± 0.19[b]	1.65 ± 0.09[b]	698.4 ± 14.2[b]

Values represent mean ± SD (n = 3). Values with different superscripts differ significantly at $p < 0.05$.

The results demonstrate that moderate auxin concentration enhanced cellular proliferation and tyrosinase synthesis, whereas higher auxin levels slightly reduced enzyme activity and metabolite production.

4.3. Statistical Analysis

Table 6 One-way ANOVA for L-DOPA production at different substrate concentrations

Source of variation	SS	df	MS	F-value	p-value
Between groups	182456.2	2	91228.1	187.34	< 0.001
Within groups	4872.3	6	812.05		
Total	187328.5	8			

All experiments were conducted in triplicate, and results are expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test at $p < 0.05$.

The ANOVA results indicated highly significant differences in L-DOPA production among substrate concentrations ($p < 0.001$). Tukey's post hoc analysis revealed that 5 mM substrate concentration showed significantly higher conversion efficiency than 10 mM and 15 mM concentrations.

The decrease in conversion efficiency at higher substrate levels suggests substrate saturation and possible inhibition of tyrosinase-mediated conversion.

Table 7 Tukey's post hoc analysis for the effect of 2,4-D concentration on L-DOPA production

Comparison	Mean difference (mg L^{-1})	p-value	Significance
0.5 vs 1.0 mg L^{-1}	373.3	0.002	Significant
1.0 vs 1.5 mg L^{-1}	87.2	0.041	Significant
0.5 vs 1.5 mg L^{-1}	286.1	0.008	Significant

The statistical analysis confirmed that 2,4-D at 1.0 mg L^{-1} produced significantly higher L-DOPA levels compared to both lower and higher concentrations.

Pearson correlation analysis showed a strong positive correlation between tyrosinase activity and L-DOPA production ($r = 0.94$, $p < 0.01$), indicating that increased enzyme activity directly enhanced the efficiency of biotransformation.

5. Discussion

The present study demonstrates the successful establishment of callus and cell suspension cultures of *Solanum tuberosum* for the biotransformation of L-tyrosine into L-DOPA, with significant optimization of cultural and biochemical parameters. The findings highlight the critical roles of plant growth regulators, enzyme activity, and substrate concentration in determining the efficiency of L-DOPA production.

The highest callus induction frequency and growth index observed in treatment T3 (NAA 2.0 mg L^{-1} + kinetin 2.0 mg L^{-1} + 2,4-D 1.0 mg L^{-1}) indicate a synergistic interaction between auxin and cytokinin in promoting cellular dedifferentiation and proliferation. Similar observations have been reported in *Mucuna* species, where balanced hormone combinations significantly enhanced callus biomass and metabolite accumulation [14]. The production of friable callus under optimized conditions is particularly advantageous, as such tissues facilitate the establishment of suspension cultures due to their loose cellular organization and high metabolic activity. The increase in growth index with moderate auxin concentration is consistent with earlier reports indicating that optimal levels of 2,4-D stimulate cell division, whereas excessive concentrations may lead to growth inhibition due to hormonal imbalance or cytotoxic effects [15].

Suspension cultures exhibited significantly higher tyrosinase activity compared to solid callus cultures, suggesting improved metabolic efficiency under liquid culture conditions. This enhancement can be attributed to better nutrient availability, aeration, and homogeneous distribution of cells in suspension systems. Similar findings have been reported in recent plant cell culture studies, where suspension cultures demonstrated superior enzymatic activity and metabolite production compared to solid cultures [16]. Since tyrosinase catalyzes the hydroxylation of L-tyrosine to L-DOPA, its elevated activity directly contributes to enhanced biotransformation efficiency. Recent studies have further elucidated the catalytic mechanism of tyrosinase, confirming that its activity is a key determinant of L-DOPA yield and stability [17]. The strong positive correlation ($r = 0.94$) observed in the present study between tyrosinase activity and L-DOPA production reinforces this relationship.

The time-course analysis revealed that L-DOPA production increased progressively and reached a maximum at 36 hours, followed by a slight decline. This pattern corresponds to the exponential growth phase of the culture, during which metabolic activity is at its peak. Similar trends have been reported in both plant and microbial systems, where maximum product accumulation occurs during active growth, followed by a decrease due to product degradation or metabolic shifts [18]. The decline in L-DOPA concentration beyond the optimal incubation period can be attributed to its oxidation to dopaquinone, a well-known phenomenon in tyrosinase-mediated reactions [17]. Recent reports have

also indicated that prolonged incubation may lead to enzymatic over-oxidation and reduced product stability, thereby lowering overall yield [19].

The effect of substrate concentration revealed that increasing L-tyrosine levels enhanced L-DOPA production up to a certain limit, beyond which conversion efficiency declined. This observation is consistent with classical enzyme kinetics, where enzyme saturation occurs at higher substrate concentrations, leading to reduced catalytic efficiency. Similar results have been reported in *Mucuna pruriens* cell cultures, where precursor feeding improved L-DOPA production, but excessive substrate concentrations resulted in decreased conversion efficiency due to metabolic constraints and possible substrate inhibition [20]. Additionally, high substrate levels may lead to feedback inhibition or accumulation of intermediate metabolites, further affecting enzyme performance. As shown in Table 4, increasing the initial L-tyrosine concentration from 1 mM to 15 mM led to a proportional rise in L-DOPA production, from 102.4 mg L^{-1} to 1525.7 mg L^{-1}. More importantly, the conversion efficiency also improved steadily from 20.5% to 90.2%, indicating that the enzyme system does not suffer substrate inhibition within this range. The low standard deviations and distinct superscript letters confirm that all increases in yield are statistically significant ($p < 0.05$). These results suggest that higher substrate concentrations, particularly 15 mM, are optimal for maximizing both L-DOPA titer and substrate utilization efficiency in this bioprocess. Further increases beyond 15 mM should be investigated to determine if the trend continues or if inhibition eventually occurs [21].

The concentration of 2,4-D significantly influenced callus growth, tyrosinase activity, and L-DOPA production, with 1.0 mg L^{-1} yielding the best results. This finding supports previous reports that moderate auxin levels enhance both cellular proliferation and secondary metabolite synthesis, while higher concentrations may suppress enzyme activity and metabolite accumulation [14,20]. The observed reduction in L-DOPA production at higher auxin levels may be attributed to altered gene expression or metabolic flux, which can negatively impact enzyme synthesis and activity.

Statistical analyses confirmed the significance and reliability of the observed differences among treatments. The results of ANOVA and Tukey's post hoc test demonstrated that variations in substrate concentration and growth regulator levels significantly affected L-DOPA production. The strong positive correlation between tyrosinase activity and L-DOPA production further emphasizes the central role of enzymatic activity in determining biotransformation efficiency. Recent studies have also highlighted the importance of enhancing enzyme activity through elicitation, precursor feeding, and metabolic engineering approaches to improve L-DOPA yield [20,22].

In comparison with other production systems, plant tissue culture offers a sustainable and controlled approach for L-DOPA production. Studies on *Mucuna pruriens* and other plant species have reported enhanced yields through elicitation and optimization strategies [22]. The yields obtained in the present study are comparable to or higher than several previously reported plant-based systems under non-elicited conditions. Although microbial and synthetic biology approaches have recently achieved higher productivity, they often involve complex optimization and may lack the stereospecific advantages of plant-derived L-DOPA [23, 24]. Therefore, plant cell culture systems continue to represent a promising platform for the efficient and scalable production of L-DOPA.

6. Conclusion

This study demonstrates that cell suspension cultures of *Solanum tuberosum* provide an efficient system for L-DOPA production from L-tyrosine. Optimized combinations of growth regulators and culture conditions enhanced callus formation, tyrosinase activity, and biotransformation efficiency. Maximum production was achieved under optimal substrate concentration and incubation time, while higher substrate levels reduced conversion efficiency due to possible enzyme saturation.

The results highlight the potential of plant cell cultures as a sustainable platform for L-DOPA production. Further work should focus on the isolation, purification, and characterization of L-DOPA using techniques such as HPLC, along with process optimization and scale-up for industrial applications.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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