Alkaloid Production in Callus of *Polyalthia bullata* and Its Potential in Metabolic Engineering

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INTRODUCTION

Alkaloids are group of nitrogen containing compounds that are derived from plant secondary metabolism. The genus of *Polyalthia*, which includes *Polyalthia bullata* (Tongkat Ali Hitam) has been reported rich in alkaloids. The presence of three alkaloids (7,7'-bisdehydro-O-methylisopiline, 7-dehydronornuciferine-7-dehydro-O-methylisopiline, and urabaine) was reported in stem. However, the production of alkaloids in plant is usually low, and some of them are tissue-specific and growth stage-specific. The used of callus culture are believed can enhance the alkaloid production in *P. bullata* even though in some cases, the production of alkaloids was still low. Hence, further approaches through alteration of alkaloid biosynthesis pathways by diverting common precursors, enzymes, and regulatory proteins can be done in enhancing alkaloid production in callus of *P. bullata.*

PROBLEM STATEMENT

The numerous benefits of alkaloid compounds might become one of the reasons of overcollection of *P. bullata* from the forest.

OBJECTIVE

To enhance alkaloid production in callus of *P. bullata* by manipulating the culture medium using auxins, elicitors, and precursors.

MATERIALS AND METHODS

Polyalthia bullata

Callus culture

Friable *P. bullata* callus

Auxins
Elicitors
Precursors

Extraction of alkaloid

Total alkaloid content

Low alkaloids

Metabolic engineering

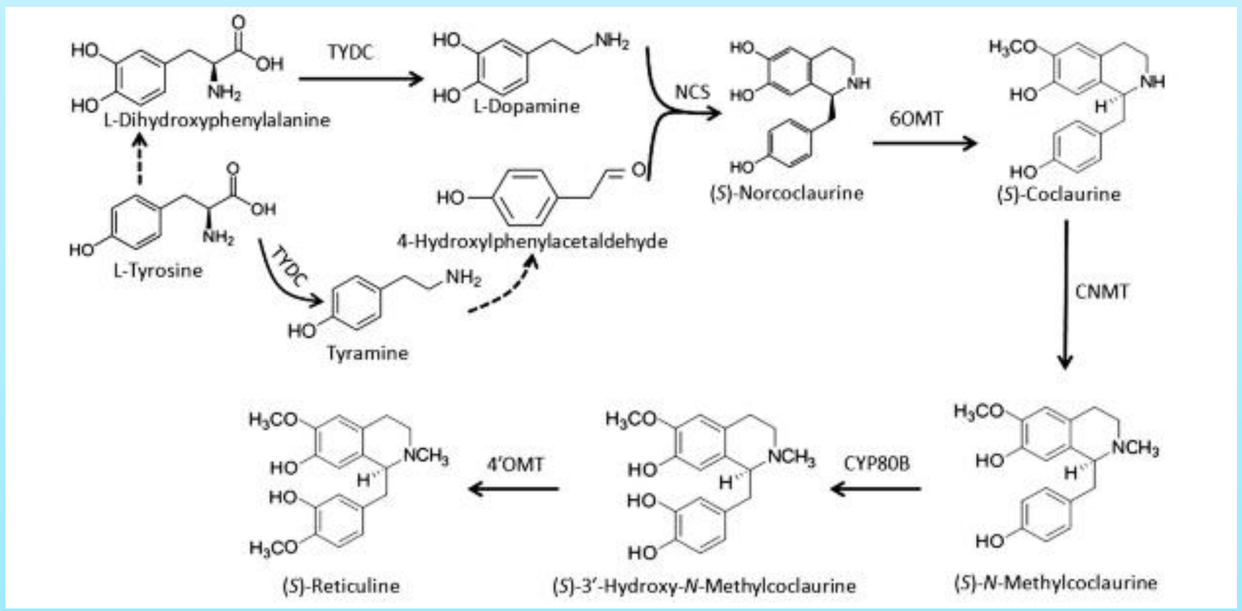
RESULTS AND DISCUSSION

Alkaloids detected in different tissues of *P. bullata*

P. bullata tissues	Group of alkaloids
Leaf	Pyridine Indole Acridone Indeno
Stem	Benzyltetrahydroisoquinoline Azonine Quinoline
Root	Indole Oxoaporphine Isoquinoline

Table 1: Group of alkaloids detected *P. bullata* leaf, stem, and root

- Based on the results, the major alkaloid compounds detected was from the class of benzylisoquinoline alkaloid (BIA).



Total alkaloid content in leaf, stem, root and callus of *P. bullata*

P. bullata samples	Total alkaloid content (µg/mg DW)
Leaf	4.61 ± 0.04
Stem	7.71 ± 0.04
Root	7.63 ± 0.06
Callus treated with 30 µM 2,4-D	31.03 ± 0.05
Callus treated with 30 µM 2,4-D + 50 µM chitosan	31.30 ± 0.23*
Callus treated with 30 µM 2,4-D + 150 µM tyrosine	10.21 ± 0.23

Table 2: The total alkaloid content recorded in methanolic extracts of *P. bullata* leaf, stem, root, and callus supplemented with the best concentrations of auxins, elicitors, and precursors. The asterisk (*) represents the highest total alkaloid content.

- The use of callus culture was capable in enhancing alkaloid production in *P. bullata* plant.
- Callus culture has been reported to have the ability to synthesize secondary metabolites and it enables the manipulation of secondary metabolites biosynthesis pathways (Chandran et al., 2020).
- Addition of elicitors like chitosan, salicylic acid, and methyl jasmonate has been reported to trigger secondary metabolic pathway to induce plant defense response to protect the cell.

Figure 1: Proposed pathway of common precursor to BIA biosynthesis in plants starting from tyrosine. TYDC, tyrosine/DOPA decarboxylase; NCS, norcoclaurine synthase; 6OMT, norcoclaurine 6-O-methyltransferase; CNMT, (S)-coclaurine N-methyltransferase; CYP80B, (S)-Nmethylcoclaurine 3'-hydroxylase; 4'OMT, 3'-hydroxy-N-methylcoclaurine 4'-O-methyltransferase (Source: Deng et al., 2018)

- The biosynthesis of BIA was reported to start by decarboxylation of tyrosine or L-dihydroxyphenylalanine (DOPA) resulting in formation of 4-hydroxyphenylacetaldehyde (4-HPAA) and L-dopamine, respectively, under the catalyzation of tyrosine/DOPA decarboxylase (TYDC) (Deng et al., 2018).
- (*S*)-reticuline is a common precursor for formation of different BIA classes (Desgagné-Penix & Facchini, 2012).

CONCLUSION

- The *P. bullata* callus showed the higher alkaloid production as compared with wild *P. bullata* plant.
- The increment of alkaloids in callus was detected when elicitors and precursors were added into the nutrient media, but the production of alkaloids was still low.

FUTURE RECOMMENDATION

The metabolic engineering of alkaloids in callus of *P. bullata* can be carried out to enhance the alkaloid production. Overexpression or down regulation of metabolic pathways by diverting common precursors, enzymes, and regulatory proteins can be the options.

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