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Research Article

Upregulation of Glucomannan Synthesis Genes in Porang (*Amorphophallus muelleri* Blume) after Polyploidy Induction using Colchicine

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ABSTRACT

Inducing polyploidy in porang using colchicine has been explored and successfully enhanced the genetic variability of porang. However, the specific impact of polyploidy on the expression of the genes responsible for glucomannan synthesis in porang has not been extensively studied. Therefore, this study aims to evaluate the effect of polyploidy induction on the expression of genes responsible for glucomannan synthesis in porang. Porang shoot explants (2n = 26) were used for polyploidy induction. Polyploidy induction was carried out using colchicine added to the culture medium. The concentrations of colchicine used in this study were 0 %, 0.01 %, 0.02 %, 0.03 %, and 0.04 %. After 53 days in media without colchicine, explants were collected for flow cytometry analysis and RNA isolation. The application of colchicine at various concentrations induced tetraploid and mixoploid porang. Mutation induction using colchicine also increases the expression (upregulation) of the CSLA, GMPP, CSLD, and GMPP genes encoding glucomannan biosynthesis in porang. Tetraploid (0.01 % colchicine treatment) and mixoploid porang (0.02 % colchicine treatment) showed the highest expression of genes involved in glucomannan biosynthesis via both sucrose and invertase pathways.

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INTRODUCTION

Porang (*Amorphophallus muelleri* Blume) is a tuberous plant that belongs to the Araceae family. This tuberous plant is valued for its high content of glucomannan, a polysaccharide with various industrial (Zhang et al. 2022) and pharmaceutical applications (Ahmadi et al. 2022). Due to its versatile applications in the food, pharmaceutical, and industrial sectors, porang exports have steadily increased in recent years (Smith & Rahardjo 2021). This is also the result of the development of porang cultivation, both through intensification and extensification by the Indonesian government (Irianto et al. 2023). However, the continued use of porang seedlings only from the same source poses a risk to future porang cultivation because of reduced genetic diversity (Wahyudi et al. 2013), leading to vulnerability to pathogen attacks. Therefore, genetic modifications aimed at increasing genetic diversity, especially increasing glucomannan content, are needed.

Increasing glucomannan synthesis can be achieved via polyploidy induction. Polyploidy is a common phenomenon in plants (Anatskaya et al. 2022) and can lead to significant changes in their genetic (Manzoor et al. 2019) and physiological characteristics (Sabzehzari et al. 2019) and is also important in plant genetics and breeding (Touchell et al. 2020). Polyploidy induction has been reported to enhance the physiological activity of *Salvia officinalis*, leading to increased stress tolerance and higher content of secondary metabolites (Hassanzadeh et al. 2020)

The induction of polyploidy in porang using colchicine has been explored and shown to successfully enhance its genetic variability (Suyono et al. 2023). However, the specific impact of polyploidy on the expression of the genes responsible for glucomannan synthesis in porang has not been extensively studied. Previous research suggests that polyploidization can increase the overall metabolic activity in plants (Corneillie et al. 2019; Mohammadi et al. 2023), potentially influencing the biosynthesis of important compounds such as glucomannan.

The biosynthesis of konjac glucomannan (KGM) involves multiple steps, where various enzymes transform substrates such as sucrose into glucomannan. The process begins with photosynthates from the leaves transported to the tubers in the form of sucrose (Gille et al. 2011). Sucrose is then converted into glucomannan through the invertase (INV) pathway and the sucrose synthase (SUS) pathway. The sucrose pathway involves the cellulose synthase-like D (CSLD) and UDP-glucose pyrophosphorylase (UGP) genes, while the invertase pathway involves the cellulose synthase like-A (CSLA) and GDP-mannose pyrophosphorylase (GMPP) genes in its biosynthesis (Diao et al. 2014).

Detecting gene expression for glucomannan synthesis via sucrose and invertase pathways has been applied in various glucomannan-producing plants (Diao et al. 2014; Wu et al. 2021; Gao et al. 2022). High expression levels of CSLA, CSLD, UGP, and GMPP were observed during developmental stages 2 and 3 in *Amorphophallus konjac*, which are believed to play a major role in KGM biosynthesis (Gao et al. 2022). The enzyme UDP-glucose pyrophosphorylase (UGP) catalyses the formation of UDP (uridine diphosphate)-glucose, which functions as a substrate and glycosyl donor in the synthesis of β-glucan for the glucomannan chain (Yang et al. 2020). GDP-mannose pyrophosphorylase (GMPP) synthesises GDP-mannose, which is then transported to the Golgi apparatus and used as a substrate for the formation of CSLA and/or CSLD (Qi et al. 2022). Subsequently, the CSLD and CSLA genes encode the glycosyltransferase enzyme that synthesises β-1,4 glucan from UDP -glucose to form the main chain of cellulose and hemicellulose (glucomannan) (Yang et al. 2020). Therefore, CSLA, CSLD, UGP, and GMPP are commonly used to identify KGM biosynthesis in *Amorphophallus*.

Understanding the expression of genes related to glucomannan synthesis is important for evaluating the success of mutation induction using colchicine. This information is also critical for understanding the impact of induced mutations on glucomannan production, assessing the potential for improved yield or quality, and guiding further optimisation of genetic modification strategies. Therefore, this study aims to evaluate the effect of polyploidy induction on the expression of the glucomannan synthesis genes in porang (*A. muelleri* Blume).

MATERIALS AND METHODS

Research design and sample preparation

The experiment was conducted using a Completely Randomised Design (CRD) with five treatments and three replications, resulting in a total of 15 experimental units (n = 15). The factor used in this study was colchicine concentration with the following levels: 0.01 %, 0.02 %, 0.03 %, and 0.04 %. A control treatment without colchicine (0 %) was included as a comparison to evaluate the effect of colchicine application.

Porang shoot explants (2n = 26) were subcultured on Murashige and Skoog (MS) media at pH 6 with the addition of 2 mg L^{-1} benzyladenine (BA). The explants were maintained at 21 °C under 24-hour fluorescent light of 1500 lux. Explants that had been cultured for 30 days were subsequently used for polyploidy induction.

Polyploidy induction

Young shoot explants of porang (2n = 26) were selected from in vitro cultures for polyploidy induction. Polyploidy induction was carried out using colchicine added to the culture medium. The stock solution was prepared at a concentration of 1 % by dissolving 280 mg of colchicine powder in 28 mL of distilled water. This stock solution was then diluted to obtain treatment solutions with concentrations of 0 %, 0.01 %, 0.02 %, 0.03 %, and 0.04 %. Colchicine added to a bottle containing 12.5 mL of MS media using a 0.22 µm Millipore filter. After 7 days under colchicine treatment, explants were transferred to MS medium without colchicine. After 53 days in media without colchicine, explants were ready for observation.

Ploidy level estimation using flow cytometry

A total of 25 mg of porang leaf explant was ground in a mortar containing 100 µL of LBO1 lysis buffer (15 mM Tris, 2 mM Na_2EDTA, 0.5 mM spermine.4 HCl, 80 mM KCl, 20 mM NaCl, 0.1 % (vv^{-1}) Triton X-100 (pH 8)). The mixture was then incubated for 5 minutes at room temperature and filtered using a 30 µm nylon mesh to isolate the nuclei (Parsons et al. 2019). The filtrate was stained with 5 µL of propidium iodide fluorescent dye solution (50 µg mL^{-1}) (Ermayanti et al. 2018) and 2.5 µL RNAse (20 mg mL^{-1}). The solution was then incubated on ice for 15 minutes and homogenised by vortexing. A of 15 sample was then analysed using a flow cytometer (Guava easyCyte), and the data were analysed using InCyte 3.1 software.

RNA Isolation and RT qPCR analysis

53-day-old explants were used for RNA extraction. Total RNA was extracted using RNAprep Pure Plant Plus Kit TIANGEN following the manufacturer's instructions. cDNA synthesis was performed using NEXscript RT 2X Master Mix. The mixture for cDNA synthesis consisted of 4 µL ddH2O, 1 µL forward primer (50 µM), 1 µL reverse primer (50 µM), 4 µL RNA template, and 10 µL NEXscript RT 2X Master Mix. The mixture was then incubated at 53 °C for 45 minutes and continued at 95 °C for 5 minutes. The incubation was performed on a thermal cycler.

The RT-qPCR procedure was carried out following the HRM Analysis Kit (Evagreen) protocol from TIANGEN. The reaction system was prepared according to the protocol guide for the HRM Analysis Kit. The primers used for the RT-qPCR are listed in Table 1. The RT-qPCR procedure was carried out using a Bio-Rad CFX 96 with an initial denaturation at 95 °C for 2 minutes, denaturation of 95 °C for 10 seconds, and annealing 55-60 °C (Table 1) for 30 seconds, repeated for 40 cycles. The gene elf-4a served as an internal control.

Table 1. Primer sequence used for detection of glucomannan activity genes.

Primer	Sequence	Annealing (°C)
CSLD (Gao et al. 2022)	F: (5'-CGACTTCAAGATCTGCACCG-3') R: (5'-CTTCACCACCTCCTCCATGT-3')	58 °C
UGP (Coleman et al. 2007)	F: (5′-GCAACTTCAGATCTGCTTCTTG-3′) R: (5′-TCCAATTTCACACCAGATTTTG-3′)	56 °C
CSLA 2 (Gao et al. 2022)	F: 5'-TGCTGAGGAGTGCTTGATGA-3' R: 5'-TGAAGCCGAAGAATGCATGG-3'	60 °C
GMPP 1 (Gao et al. 2022)	F: 5'-CTCCAGCAGTATCATCGGGT-3' R: 5'-AAATCTCATCGCCCACATGC-3'	60 °C
elF-4a (Wang et al. 2017)	F: 5'-ACAAGATGAGGAGCAGGG-3' R: 5'-GGTGATAAGGACACGAGA-3'	55 °C

Statistical analysis

Flow cytometry data were analysed descriptively with InCyte 3.1 software. to observe the distribution of polyploid cells. Ploidy level was determined by comparing the peak curves between the control and the mutated porang. Gene expression analysis was carried out using Bio-Rad CFX Maestro 96 software with the Livak method (delta-delta Ct method). The relative expression of target genes (CSLD, UGP, CSLA2, and GMPP) was normalised to the housekeeping gene elf-4a. Analysis of variance (ANOVA), followed by Duncan test 95 % confidence, was performed on the relative expression data of target genes.

RESULTS AND DISCUSSION

Effect of colchicine on ploidy level

The application of colchicine with various concentrations (0 %, 0.01 %, 0.02 %, 0.03 %, and 0.04 %) increased the ploidy level of porang explants (*A. muelleri*) (Figure 1). Colchicine treatment induced the formation of both tetraploid (4n = 4x = 52) and mixoploid porang, in contrast to the diploid control (2n = 2x = 26) (Figure 1). However, most colchicine treatments resulted in mixoploid porang. Tetraploid porang was observed after 0.01 % colchicine treatment, whereas mixoploid porang resulted after 0.02 %, 0.03 %, and 0.04 % of colchicine treatment (Figure 1).

Colchicine treatments of 0.01-0.05 % are commonly used concentration and have been shown to produce tetraploid plants (Nilanthi et al. 2008; Wang et al. 2024). However, the response to colchicine differs depending upon the plant. For example, 0.01 % colchicine treatment successfully produced tetraploids in *Lilium regale* (Jeloudar et al. 2019), but 0.5 % colchicine is needed to induce tetraploid in *Trichosanthes dioica* (Hassan et al. 2020). Conversely, higher concentrations of colchicine have been associated with phytotoxic effects and reduced explant viability (Heo et al. 2016), underscoring the importance of optimising colchicine dosage for each species.

Several studies have confirmed the effectiveness of colchicine in producing polyploid plants with desirable traits. In fruit crops like blackberry (Sabooni et al. 2022) and bananas (Amah et al. 2019), colchicine treatment

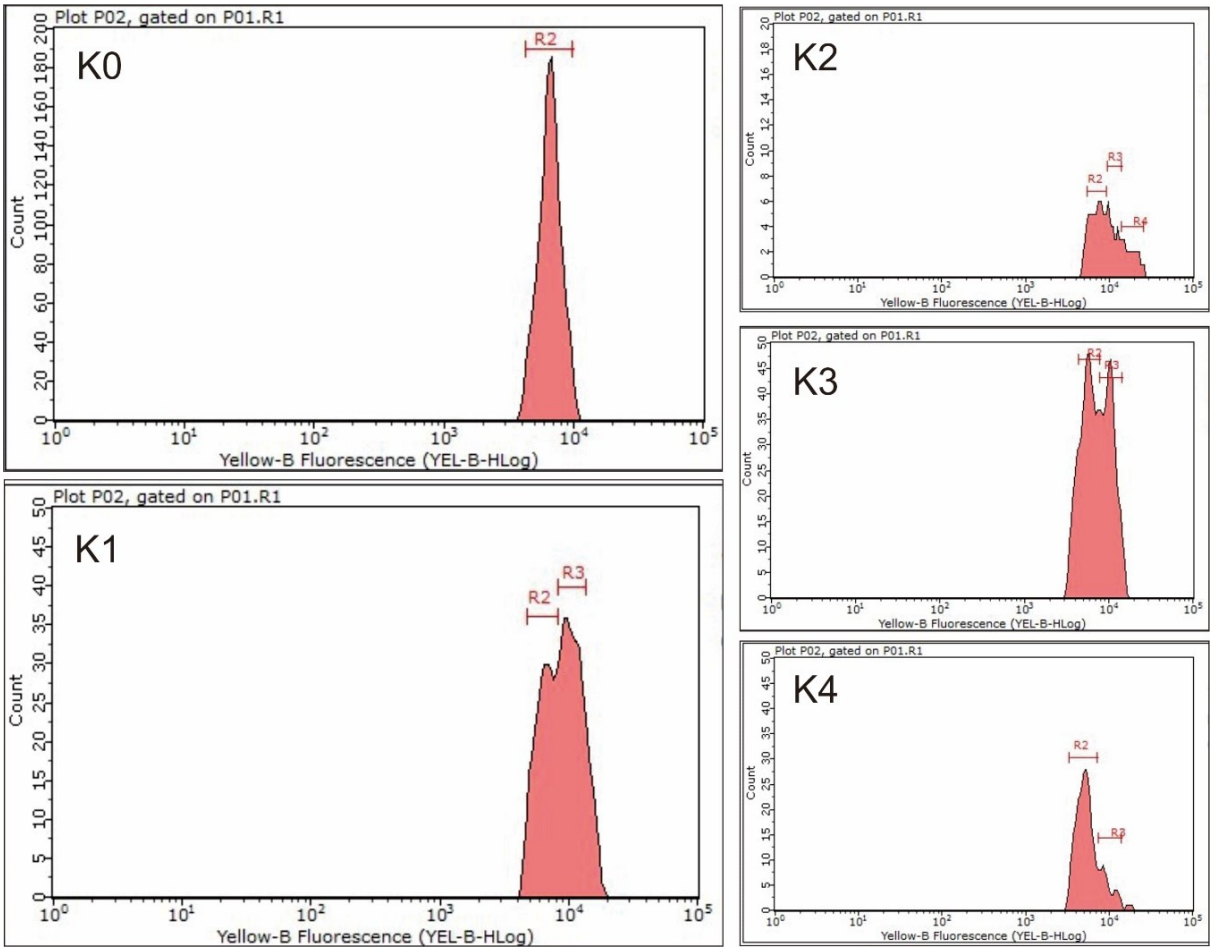

Figure 1. Ploidy category of porang (*A. muelleri*) after colchicine induction. K0: untreated porang (diploid peak (R2) of control porang close to channel 10000), K1: tetraploid porang after 0.01 % colchicine induction, K2: mixoploid porang after 0.02 % colchicine induction, K3: mixoploid porang after 0.03 % colchicine induction, K4: mixoploid porang after 0.04 % colchicine induction.

affects morphological characteristics and improves flavour profiles due to increased cell size and altered metabolic pathways. In ornamental plants, colchicine-induced polyploidy has enhanced flower size, colour intensity, and resistance to environmental stressors (Manzoor et al. 2019). In addition, colchicine-induced polyploidy also successfully changes the morphological characteristics (Widoretno et al. 2023) and genetic variability (Suyono et al. 2023) of porang.

Overall, colchicine is an effective mutagenic agent for chromosome doubling, providing agronomic benefits such as increased plant organ size and higher yield potential. However, despite its effectiveness, one significant challenge in its application is the occurrence of mixoploidy (Figure 1) (concentration 0.2-0.4 %). Mixoploidy refers to the presence of cells with different ploidy levels within the same organism, which can lead to uneven growth, developmental anomalies, and inconsistent trait expression (Marisa et al. 2012). This condition arises because colchicine treatments do not always result in uniform chromosomal duplication across all cells, particularly when the treatment duration, concentration, or method of application is not carefully optimised. This phenomenon occurs not only in poran but also in *Neolamarckia cadamba* (Eng et al. 2021), *Brassica* and *Capsicum annuum* (Tammu et al. 2021), where mixoploid plants were produced after colchicine induction.

Mixoploidy is a common phenomenon in polyploidy induction using chemical agents such as colchicine (Tammu et al. 2021). In this study, colchicine concentrations of 0.02–0.04 % produced mixoploid plants, indicating partial success in chromosome duplication. However, the ploidy stability aspect of mixoploid plants has not been further studied. Mixoploidy stability is im-

portant to ensure that the induced genetic changes remain consistent during growth, regeneration, and both generative and vegetative reproduction. Mixoploid plants are also at risk of somatic instability, which is a change in ploidy proportions between tissues or over time due to uneven cell division (Marisa et al. 2012; Mehravi et al. 2022). In addition, selection of superior plants becomes difficult because gene expression and metabolite production can vary between plant parts (Marisa et al. 2012; Eng et al. 2021). Therefore, further evaluation of ploidy distribution between tissues and trait inheritance during the generative phase is crucial.

Although mixoploidy presents several limitations, it also holds potential applications, especially if it can be utilised vegetatively in cultivation systems. Several studies have shown that mixoploid plants can exhibit certain advantages, including tolerance to abiotic stresses such as drought and salinity (Mehravi et al. 2022). The presence of both diploid and polyploid cells may allow these plants to better cope with environmental challenges, thus increasing their survival rates under adverse conditions (Mondin et al. 2018). Mixoploid *Neolamarckia cadamba* has been shown to produce larger stomata and increased leaf area compared to its diploid counterparts, which can enhance photosynthetic efficiency and overall plant productivity (Eng et al. 2021). In addition, secondary metabolite production is higher in tissues with higher ploidy (Mohammadi et al. 2023).

In the context of porang, the vegetative cultivation system using tubers and bulbils (A'yun et al. 2019) allows for the practical use of mixoploids, as long as ploidy stability is maintained throughout the growth cycle. For this reason, it is necessary to carry out periodic ploidy monitoring using flow cytometry on various tissues, phenotypic evaluation under different field conditions to determine the consistency of growth, yield, and glucomannan content, and development of vegetative propagation protocols from tissues with dominant ploidy to produce stable and uniform clones (Barron et al. 2020).

Colchicine-induced gene expression involved in glucomannan synthesis

The expression of genes involved in glucomannan biosynthesis in this study was analysed in relation to the sucrose (SuS) and invertase (INV) metabolic pathways. Cellulose Synthase-Like D (CSLD) and UDP-glucose pyrophosphorylase (UGP) were involved in the sucrose pathway, whereas Cellulose Synthase Like-A (CSLA) and GDP-mannose pyrophosphorylase (GMPP) were associated with the invertase pathway. Colchicine induction with various concentrations (0.01 %, 0.02 %, 0.03 %, and 0.04 %) affected the activity of glucomannan synthesis via both sucrose (CSLD, UGP) and invertase (CSLA and GMPP) pathways in porang (*A. muelleri* Blume) (Figure 2). The increased expression of glucomannan biosynthesis genes observed after colchicine induction may be associated with ploidy changes in porang, specifically tetraploidy at 0.01 % and mixoploidy at 0.02 %.

Polyploid induction using colchicine increases the expression (upregulation) of the CSLA, UGP, CSLD, and GMPP genes involved in glucomannan biosynthesis in porang (*A. muelleri* Blume). However, polyploid induction using 0.01 and 0.02 % colchicine results in higher expression of genes related to glucomannan synthesis than other treatments (Figure 2). These results suggest that polyploid induction using colchicine at concentrations of 0.01 % (K1) and 0.02 % (K2) significantly enhances the expression of genes involved in glucomannan biosynthesis, namely CSLA, GMPP, and CSLD. The increased gene expression indicates a potential link between polyploidy and enhanced metabolic activity, leading to higher glucomannan accumulation in *A. muelleri*.

Furthermore, the elevated expression levels observed in K1 and K2 treatments suggest that chromosomal doubling may positively influence the

gene expression responsible for glucomannan synthesis. This effect could be attributed to increased gene dosage and regulatory changes in the expression of key biosynthetic enzymes (Madani et al. 2021). Comparing the different colchicine treatments, it is evident that the polyploidised plants exhibit higher expression of genes for glucomannan biosynthesis, which may have significant implications for breeding and commercial cultivation.

Colchicine treatment has also been shown to enhance glucomannan synthesis in various organisms, particularly in fungi and plants. In *Cunninghamella*, colchicine at concentrations up to 20 ppm accelerated sugar absorption and polysaccharide accumulation, indicating a potential increase in glucomannan synthesis (Salama & Naguib 1963). In Arabidopsis, the cellulose synthase-like A (CSLA) family, particularly CSLA2, CSLA3, and CSLA9, are crucial for glucomannan synthesis, with overexpression leading to increased glucomannan content (Goubet et al. 2009). Furthermore, the genome of *Amorphophallus konjac* reveals key genes involved in glucomannan biosynthesis, suggesting that genetic manipulation could further enhance production (Gao et al. 2022). These findings collectively suggest that colchicine may stimulate glucomannan synthesis through both metabolic pathways and modulating gene.

Although all genes showed increased expression after polyploidy induction, differences in expression levels between genes may reflect their regulatory positions or potential rate-limiting steps in the glucomannan biosynthesis pathway. Based on RT-qPCR results (Figure 2), the expression of CSLA and GMPP tended to increase significantly more than UGP and CSLD in the K1 and K2 treatments. This suggests that the invertase pathway likely plays a more dominant role in contributing to glucomannan biosynthesis in porang. However, biochemically, GMPP plays a crucial role as a producer of GDP-mannose, the primary sugar donor in glucomannan chain synthesis by glycosyltransferase enzymes (CSLA/CSLD) in the Golgi apparatus (Qi et al. 2022). Several previous studies have also shown that GMPP activity is a critical link in controlling carbon flow towards hemicellulose and storage polysaccharide biosynthesis (Wu et al. 2021; Gao et al. 2022). Low GDP-mannose availability due to limited GMPP expression can inhibit glucomannan chain elongation, even when CSLA expression is high. Therefore, GMPP can be considered the gene with the greatest potential to be the rate-limiting step in this biosynthetic pathway. Conversely, CSLA, as the primary catalyst in β-1,4-glucomannan chain formation, also plays an essential role. However, because its expression tends to be responsive to polyploidy treatment and is higher than that of GMPP, it is unlikely to be the primary limiting point under these treatment conditions.

Polyploidy induction has also been detected to affect increasing gene expression in several plants. Increasing the ploidy level from diploid to tetraploid has been shown to increase the expression of the podophyllotoxin (PTOX) gene in the leaves and stems of *Linum album* (Javadian et al. 2017). The increase in PTOX is also correlated with unpregulated expression of several enzymes involved in PTOX biosynthesis, such as phenylalanine ammonia -lyase (PAL) and pinoresinol-lariciresinol reductase (PLR). In addition, several genes such as monoterpene synthase (MTS) and limonene synthase (LS) in *Citrus limon* have also been reported to be upregulated under tetraploid conditions. This increase aligns with the increased antioxidant activity observed in *Citrus limon* (Bhuvaneswari et al. 2020).

Although increased expression of glucomannan biosynthesis genes (CSLA, CSLD, UGP, and GMPP) after colchicine treatment in porang (*A. muelleri*) was detected in this research, the possible involvement of epigenetic regulation is also worth considering. One of the major epigenetic changes resulting from polyploidy is DNA methylation. In the autotetraploid plant

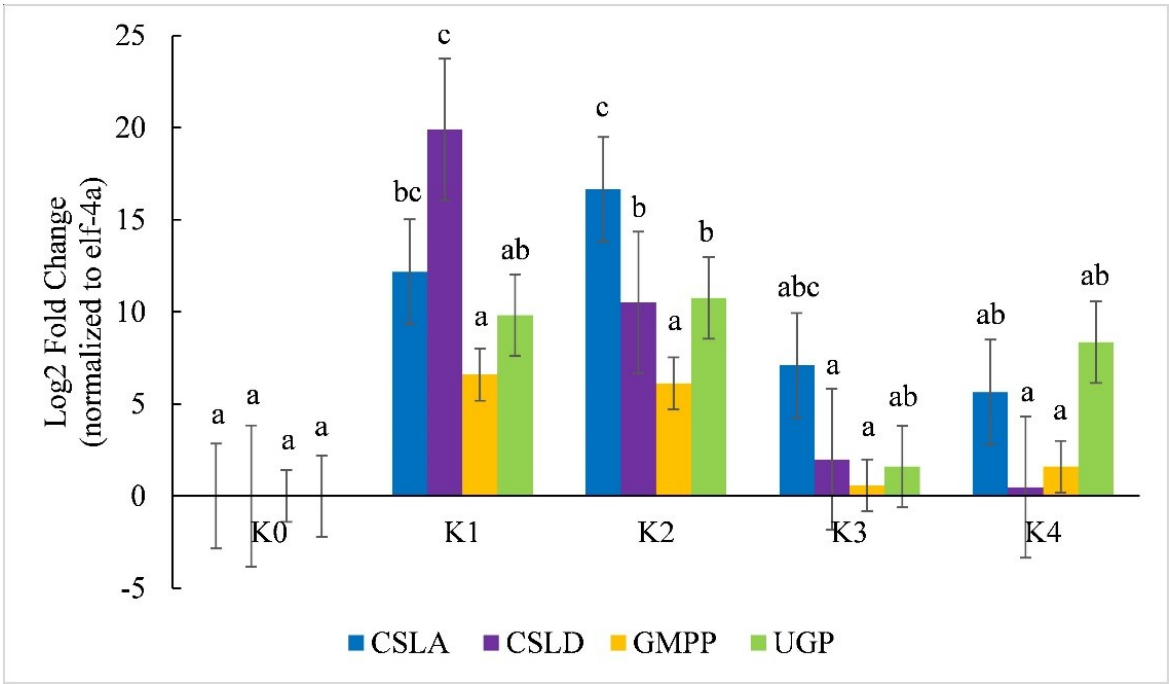

Figure 2. An increase in gene-related glucomannan synthesis after polyploid induction using colchicine. K0 = 0.00 %; K1 = 0.01 %; K2 = 0.02 %; K3 = 0.03 %; and K4 = 0.04 %. Different letters (a, b, c, d) indicate significant differences among groups ($p < 0.05$) based on ANOVA followed by Duncan test.

Brassica rapa, increased methylation was detected primarily at transposable elements (TEs) and gene regulatory regions (promoters and downstream regions), which can stabilise gene expression and prevent abnormal expression following "genomic shock" (Ma et al. 2025). Therefore, to test this hypothesis, further studies involving DNA methylation analysis are highly recommended. In addition, quantitative analysis of glucomannan content is also important to ensure that upregulation of this gene has a real impact on glucomannan biosynthesis.

The expression of CSLA, GMPP, CSLD and GMPP genes indicates that colchicine induction affects glucomannan biosynthesis through the invertase and sucrose pathways (Figure 3). Photosynthetically derived sucrose is synthesised in the leaves and transported to the tubers, where it can enter either the invertase pathway or the sucrose synthase pathway. In the invertase pathway, fructose-6-phosphate (Fru-6-P) is isomerised by phosphomannose isomerase (PMI) to generate mannose-6-phosphate (Man-6-P). Man-6-P is then converted to mannose-1-phosphate (Man-1-P) by phosphomannomutase (PMM) and further metabolised by GDP mannose pyrophosphorylase (GMPP), which catalyses the production of GDP mannose (GDP-Man) by GDP-mannose pyrophosphorylase (GMPP). The nucleotide sugars GDP-mannose, GDP-glucose, and ADP-glucose are transported into the Golgi apparatus, where they serve as substrates for glucomannan synthesis catalysed by CSLA and CSLD enzymes (Figure 3) (Qi et al. 2022).

Another advanced analysis such as transcriptomics may be needed to observe the effect of colchicine on gene expression profiles at a more comprehensive level. This approach allows for the identification of differentially expressed genes (DEGs) that are influenced by colchicine treatment, providing insights into the molecular pathways and biological processes affected (Zhou et al. 2017). Transcriptome analysis is also capable of detecting the differential expression of thousands of genes, particularly those involved in microtubule dynamics, cell division, and stress responses (Zhou et al. 2017). Furthermore, integrating transcriptomic data with proteomic and metabolomic analyses could offer a multidimensional understanding of colchicine's impact on cellular functions.

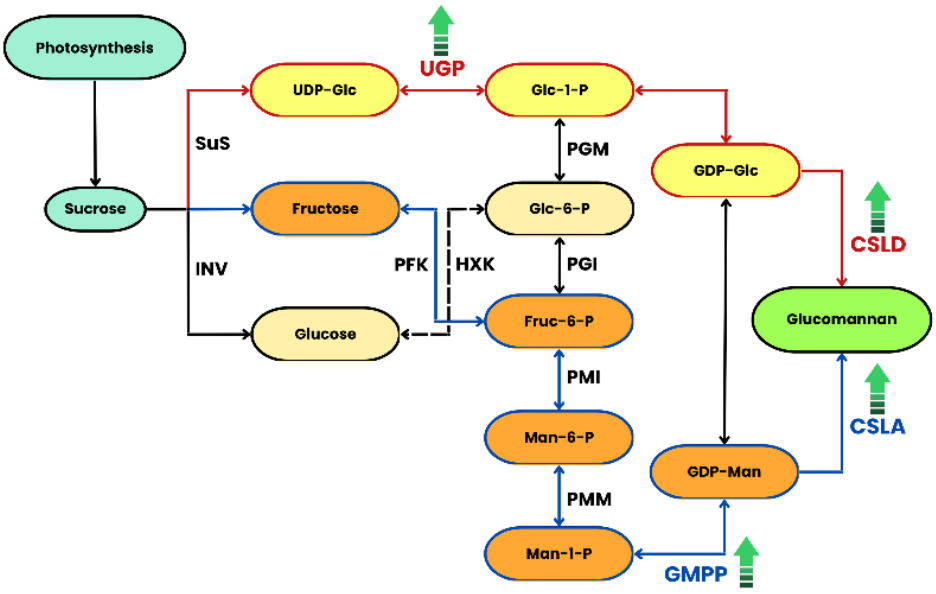

Figure 3. Pathway of glucomannan biosynthesis in porang.

CONCLUSIONS

Colchicine application in the range of 0.01 % to 0.04 % affects the ploidy level of porang explants, with 0.01 % inducing tetraploidy and higher concentrations (0.02–0.04 %) predominantly resulting in mixoploidy. Among all treatments, porang explants treated with 0.01 % and 0.02 % colchicine, resulting in tetraploid and mixoploid plants, respectively, showed the highest expression levels of genes involved in glucomannan biosynthesis through both the sucrose (CSLD, UGP) and invertase (CSLA, GMPP) pathways.

AUTHOR CONTRIBUTION

D.W. was responsible for the conceptualisation, methodology, data analysis, and writing of the original draft. S.Y.N. provided supervision, interpreted the data, and reviewed the manuscript. R.S.R. contributed to the experimental design, data collection, and manuscript review and editing. N.A.S., S.L.W. and D.R.K. conducted laboratory work and analysed data.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the research or its funding.

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