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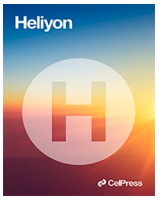

A newly identified β-amyrin synthase gene hypothetically involved in oleanane-saponin biosynthesis from *Talinum paniculatum* (Jacq.) Gaertn.

Ika Qurrotul Afifah [a], Indra Wibowo [b], Ahmad Faizal [c,*]

[a] *Chemistry Department, Faculty of Science and Technology, UIN Sunan Kalijaga Yogyakarta, Yogyakarta, 55281, Indonesia*
[b] *Physiology, Animal Development, and Biomedical Sciences Research Group, School of Life Sciences and Technology, Institut Teknologi Bandung, Bandung, 40132, Indonesia*
[c] *Plant Science and Biotechnology Research Group, School of Life Sciences and Technology, Institut Teknologi Bandung, Bandung, 40132, Indonesia*

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ABSTRACT

Talinum paniculatum or Javanese ginseng in Indonesia is a plant widely used as a traditional medicine. The genus *Talinum* produces oleanane-type saponins, such as talinumoside I. The first aim of this study was to isolate the probable gene encoding β-amyrin synthase (bAS), a key enzyme involved in the cyclization of 2,3-oxidosqualene producing the backbone of the oleanane-type saponin β-amyrin and characterize the gene sequence and the predicted protein sequence using in silico approach. The second aim was to analyze the correlation between the *TpbAS* gene expression level and saponin production in various plant organs. Thus, *TpbAS* was isolated using degenerate primers and PCR 5'/3'-Rapid Amplification of cDNA Ends (RACE), then the gene sequence and the predicted protein were in silico analyzed using various programs. *TpbAS* expression level was analyzed using reverse transcriptase PCR (RT-PCR), and saponin content was measured using a spectrophotometer. The results showed that the full-length *TpbAS* gene consists of 2298 base pairs encoding for a 765-amino acid protein. From in silico study, the (GA)n sequence was identified in the 5′-untranslated regions and predicted to be a candidate of the gene expression modulator. In addition, functional RNA motifs and sites analysis predicted the presence of exon splicing enhancers and silencers within the coding sequence and miRNA target sites candidate. Amino acid sequence analysis showed DCTAE, QW, and WCYCR motifs that were conserved in all classes of oxidosqualene cyclase enzymes. Phylogenetic tree analysis showed that *TpbAS* is closely related to other plant oxidosqualene cyclase groups. Analysis of *TpbAS* expression and saponin content indicated that saponin is mainly synthesized and accumulated in the leaves. Taken together, these findings will assist in increasing the saponin content through a metabolic engineering approach.

1. Introduction

Talinum paniculatum or "Javanese ginseng" is a widespread herbal medicine in Indonesia and other Asian regions such as Thailand. This plant leaf is commonly consumed as a vegetable supplement and the roots are used as a reproductive tonic in Thailand's

* Corresponding author.
E-mail address: afaizal@itb.ac.id (A. Faizal).

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traditional practice [1]. In addition, *T. paniculatum* or "major gomes" and "erva gorda" in Brazil is consumed and used to treat different cardiovascular disorders in folk medicine [2]. Further study to confirm the ethnomedicine of this plant extract exhibited the estrogenic activity [1], diuretic effect after prolonged exposure [2], and cardioprotective effects through serum antioxidant activity and ACE inhibition, preventing alterations of hemodynamic and endothelial function, and reducing damage to cardiac structure in a rat model [3]. This plant is classified as Unconventional Food Plants (UFP) because of the nutritional value of the leaves. The leaf insoluble dietary fiber and protein contents are within Acceptable Macronutrient Distribution Ranges (AMDR) and Adequate Intake (AI) for adults. Some of the mineral compositions meet the Dietary Reference intakes standard. The methanolic acetic acid leaf extract has antioxidant and antibacterial activity against *Bacillus cereus* [4] and the ethanol-water extract has antibacterial activity against *Serratia marcescens* and *Staphylococcus aureus* [5]. The compound mixture including triterpene saponin isolated from *T. paniculatum* leaves has antinociceptive and edematogenic activity higher than the commercial non-steroidal anti-inflammatory drugs (NSAIDs) [6].

The chemical analysis using various techniques revealed the constituents of this plant. The preliminary phytochemical screening indicated steroids, saponins, and tannins in leaf extract, steroids, triterpenes, and saponins in ethyl acetate fraction, and steroids and triterpenes in hexane fraction. The following analysis using mass spectrometry (MS) confirmed campesterol, stigmasterol, and sitosterol as major compounds [5]. The root extract of *Talinum paniculatum* contains sitosterol, stigmasterol, stigmastan-3-ol-(3β, 5α, 24S), stigmast-22-en-3-ol, and campesterol while the leaf extract contain β-sitosterol, stigmastanol, stigmasterol, and campesterol concluded from GC/MS analysis [1]. In addition, the roots of this three-month-old plant contained less saponin than roots cultivated *in vitro* for 28 days [7]. Saponin production is a defense mechanism utilized by *T. paniculatum* against biotic and abiotic stressors. Elicitation of the adventitious root with methyl jasmonate (MeJA) and salicylic acid may stimulate concentration-dependent saponin synthesis [8]. Moreover, the difference in carbon sources influences the saponin concentrations of adventitious roots. The application of 3% fructose boosted *T. paniculatum* root biomass and saponin content [9].

Saponins are non-volatile compounds commonly found in plants with various pharmacological activities reported from the *in vitro*, *in vivo*, or combination study. The *in vivo* study confirmed that saponins have anti-diabetic and anti-hyperglycemic [10], anti-stress [11], anti-inflammatory [12], anti-gastric ulcer [13], anti-tumor [14], and anti-malarial activity [15]. The antioxidants [16], and anti-hepatitis C virus [17] of saponins indicated from the *in vitro* study, and the combination study reported the anti-cancer [18], adjuvant activity enhancing the poliovirus vaccine [19], and anti-diabetic activity [20]. These potential activities can be developed to meet human needs through a concerted effort in the exploration of biological agents and drug development. The modification of natural compound structure could enhance the biological activity as indicated in the sugar moiety replacement of saponin immunoadjuvant QS-21 (acylated 3, 28-bisdesmodic triterpene glycosides (1,3)) isolated from *Quillaja saponaria* [21]. Furthermore, this compound could enhance the hydrolytic activity of β-galactosidase which is important in the food, chemical, and pharmaceutical industries to prevent lactose intolerance [22].

Conventional extraction and chemical synthesis have limitations that often hinder the needed supply to the pharmaceutical industry [23]. Metabolic engineering is a technique for modulating the production of specific compounds in plants or for building new circuits in selected microorganisms to produce increased amounts of desired compounds. However, this strategy requires knowledge of the genes encoding enzymes involved in specific metabolite biosynthesis pathways [24].

The most common plant triterpenoid saponin is an oleanane-type with a skeleton constructed of β-amyrin derived from oxidosqualene precursors through several steps mediated by β-amyrin synthase [25]. This type of saponin and its backbone has ample functions such as analgesic and anti-inflammatory, neuroprotective, Alzheimer's disease-like pathology deterrent, and antifungal against *Candida albicans* [26–29]. The molecular docking study and molecular dynamic studies suggested the potential oleanolic acid as a principal protease inhibitor of the SARS-Cov-2 [30].

Because β-amyrin synthase functions in the branch between primary and secondary metabolite pathways, characterization of β-amyrin synthase regulation could facilitate increased saponin production through metabolic engineering approaches [31]. Overexpression of the β-amyrin synthase gene and other genes encoding upstream enzymes in the saponin biosynthetic pathway are expected to increase the saponin content of *T. paniculatum*. Hyperproduction of triterpenoid saponins has been achieved through the overexpression of the squalene synthase gene in *Panax ginseng* [17]. Heterologous expression of the β-amyrin synthase from *Aster sedifolius* in *Medicago truncatula* results in increased accumulation of triterpenoid compounds compared with the controls [32]. Pathway engineering of *Escherichia coli* by introducing a set of biosynthetic pathway genes from other species, including β-amyrin synthase from *Euphorbia tirucalli* successfully produced β-amyrin [33]. Heterologous production of oleanolic acid has been conducted by the engineering of *Yarrowia lipolytica* yeast metabolism using β-amyrin synthase from *Glycyrrhiza glabra* and other genes [34]. Transformation of the β-amyrin synthase gene to crops can also be used to produce transgenic organisms with high value, such as the dammarenediol-II synthase gene from *P. ginseng* in rice. This method produces ginseng rice containing dammarane-type sapogenin [35].

To date, there have been no published studies on saponin biosynthesis in *T. paniculatum*. However, a study on *T. triangulare* identified talinumoside, an oleanane-type saponin [36]. As these two species are closely related, they may have similar biosynthetic reaction-catalyzing enzymes that produce a compound with similar structures [37]. Therefore, the β-amyrin backbone may also be present in the *T. paniculatum*.

The gene encoding β-amyrin synthase has been successfully isolated from several plant species, including *Panax ginseng* [25], *Medicago truncatula*, *Lotus japonicus* [38], *Centella asiatica* [39], *Glycine max* [40], *Aster sedifolius* [41], *Gentiana straminea* [42], *Maesa lanceolata* [43], *Bupleurum chinense* [44], and *Betula platyphylla* [45] using different types of methods. In this study, the β-amyrin synthase gene from *T. paniculatum* (*TpbAS*) was isolated using RACE PCR. The gene and predicted protein sequences were characterized using an in silico approach. Furthermore, the correlation between *TpbAS* expression and saponin production in *T. paniculatum* was also analyzed to predict the site of synthesis and accumulation.

2. Materials and methods

2.1. Plant materials

T. paniculatum obtained from Bumi Herbal Dago (Bandung, West Java, Indonesia) was used in this study. Seeds were sterilized in 96% (v/v) ethanol for 5 min and rinsed in sterile water. Seeds were germinated at 25 °C under dark conditions in Murashige and Skoog (MS) medium [46] for 7 days. Seedling materials were then propagated through axillary shoot formation and incubated in a growth chamber at 25 °C under a 16/8-h light/dark photoperiod provided by warm white fluorescence light. After 2 months, the plantlets were harvested for RNA extraction.

2.2. Molecular cloning of the β-amyrin synthase gene from T. paniculatum (TpbAS)

Total RNA was extracted from plantlets using GeneJET Plant RNA Isolation Kits (Thermo Scientific, Cat. Num. K0801) according to the manufacturer's instructions. Gel electrophoresis and spectrophotometric analyses were performed to examine the quality and concentration of RNA. Total RNA was treated with DNAse I, RNAse-free (Thermo Scientific, Cat. Num. EN0525), and used as a template for reverse transcription with a RevertAid M-MuLV Reverse transcriptase kit and oligo dT primers (Thermo Scientific, Cat. Num. K1621) in a total volume of 20 μL. A partial sequence was obtained via PCR with degenerate primers that were designed based on the highly conserved regions of other plants' β-amyrin synthase genes available in GenBank. Amplification was performed using DreamTaq Green PCR Master Mix (Thermo Scientific, Cat. Num. K1081) for 35 cycles of 30 s at 95 °C, 30 s at 50.7 °C, and 1 min at 72 °C, with a pre-denaturation of 3 min at 95 °C and final extension of 5 min at 72 °C. PCR products were examined by 1% gel electrophoresis, stained in EtBr solution, and visualized using a UV transilluminator. The band of the predicted size (660 bp) was sequenced.

Based on the partial sequence, gene-specific primers (RACE5GSP and RACE3GSP) were designed for 5′ and 3′-Rapid Amplification of cDNA Ends (RACE). RACE PCR was carried out using SMARTer® RACE 5'/3′ kit (Clontech Cat. Num. 634858) according to the manufacturer's instructions. The 5′- and 3′-RACE PCR products were cloned into a pRACE plasmid and sequenced using M13 primers. Sequences of the start and stop regions obtained from partial sequence contigs were used for coding DNA sequence (CDS) PCR primers (PFTAs_PF11 and PRTAs_PR3). CDS amplification was performed using SeqAmp DNA polymerase for 30 cycles of 30 s at 94 °C, 30 s at 68 °C, and 3 min at 72 °C with final extension 3 min at 72 °C. The product was cloned into a pRACE plasmid using the fusion method and verified by sequencing. All primer sequences used for cloning are listed in Table S1.

2.3. Sequence analysis and phylogenetic tree construction

Partial gene sequence contigs of *TpbAS* were obtained using the CLC Main Workbench 7.8.1 program. The online ORF-finder program (www.ncbi.nlm.nih.gov) was used to detect open reading frames. The molecular weight and theoretical isoelectric point were calculated using the ProtParam tool at http://web.expasy.org/protparam/. Alignment of the amino acid sequence of TpbAS and β-amyrin synthase sequences from other plant species for motif analysis was performed using T-Coffee at http://tcoffee.crg.cat/apps/tcoffee/do:regular. The BoxShade Server at http://www.ch.embnet.org/software/BOX_form.html was then used to generate publication-quality alignment. Functional RNA motifs and sites were also identified using an integrated web server RegRNA 2.0 at http://regrna2.mbc.nctu.edu.tw [47]. A Maximum Likelihood tree was constructed using MEGA version X [48] with bootstrapping 1,000× for reliability evaluation. The Jones-Taylor-Thornton (JTT) model was used for estimating the number of amino acid substitutions. In this step, several amino acid sequences of β-amyrin synthase, lupeol synthase, cycloartenol synthase, lanosterol synthase, dammaranediol synthase, cucurbitadienol, and mixed-amyrin synthase deposited in GenBank from different plant species and other organisms with accession number presented in Table S3 were used. Lanosterol synthase from *Saccharomyces cerevisiae* was used as an outgroup.

2.4. Characterization of TpbAS gene expression in different tissues of T. paniculatum

RNAs isolated from leaves, stems, and roots were used as the template for cDNA synthesis using a RevertAid First Strand cDNA Synthesis kit (Thermo Scientific, K1621). *TpbAS* and housekeeping gene *NADH*, were amplified via PCR. Amplification was performed using DreamTaq Green PCR Master Mix (Thermo Scientific, K1081) for 35 cycles of 30 s at 95 °C, 30 s at 56 °C, and 1 min at 72 °C, with a pre-denaturation step of 3 min at 95 °C and a final extension of 5 min at 72 °C. PCR products were examined by gel electrophoresis (1.5%), stained in EtBr solution, and visualized using a UV transilluminator.

2.5. Analysis of saponin production in different tissue of T. paniculatum

Leaves, stems, and roots of *T. paniculatum* were ground in liquid nitrogen. Approximately 50 mg of sample powder was extracted with 250 μL pure MeOH, sonicated for 3 h at room temperature, and centrifuged at 6462 g for 5 min. The upper phase was separated from cell debris and evaporated overnight in a fume hood. The crude extracts were dissolved again in 250 μL MeOH for concentration measurements. Samples were reacted with a Lieberman Burchards (LB) reagent (glacial acetic acid and concentrated sulfuric acid; 1:5, v/v), incubated for 10 min at room temperature, and then the absorbance was measured at wavelength 580 nm [49]. Oleanolic acid was used as a standard. The obtained data were then analyzed using one-way ANOVA followed by Duncan's multiple range test. The

statistical analysis was performed at the level of *P*-value less than 0.05 using SPSS 16.0 (SPSS Inc. USA).

3. Results

3.1. Cloning of TpbAS

A partial sequence (660 bp) of *TpbAS* was obtained by PCR using degenerate primer sets of BasParF2 and BasParR2 (Table S1) that were designed based on a highly conserved region of β-amyrin synthase genes from several plants deposited in GenBank. Alignment analysis showed that the gene has 79% identity with the β-amyrin synthase gene of *Vaccaria hispanica*. 5'/3'-RACE PCR was performed to obtain the CDS of *TpbAS* using the specific primers that were designed based on the partial sequence. The CDS predicted from ORF analysis consisted of 2298 bp encoded 765 amino acids with a calculated molecular mass of 88,102 kDa and a theoretical isoelectric point of 6.05. The total number of negatively charged residues (Asp + Glu) was 92 and the positively charged residues (Arg + Lys) was 78. The deduced amino acid sequence contained QW, DCTAE, and WCYCR motifs (Fig. S1) that are highly conserved among the known oxidosqualene cyclases from plants. The sequence data of *TpbAS* has been deposited in GenBank with accession number MG492000 at 29-AUG-2018.

5'/3'-RACE PCR was successfully used to amplify several sequences of the 5'- and 3'-untranslated regions (UTR) of *TpbAS*. Furthermore, (GA)n sequence was identified in the region upstream of the ATG start codon as displayed in Fig. 1.

Functional RNA motifs and sites analysis showed hexa, hepta, and octanucleotide sequences within TpbAS coding sequence and were identified as exon splicing enhancers (ESE) candidate previously discovered in another kingdom (Fig. 2 and Table S2). Several microRNA target site candidates were also discovered in the coding sequence (Fig. 2).

3.2. Phylogenetic analysis of TpbAS

Alignment of *TpbAS* with other genes in GenBank indicated that this gene has a high degree of identity with β-amyrin synthases from other plants, including *Barbarea vulgaris*, *V. hispanica*, and *Chenopodium quinoa* with 87%, 86%, and 86% identity, respectively [50]. These identities confirmed that the gene obtained belongs to the β-amyrin synthase group. In addition, *TpbAS* has a 70% degree of identity with lupeol synthase from *Theobroma cacao*. The phylogenetic tree showed that TpbAS is closely related to β-amyrin synthases from other plants and other oxidosqualene cyclase enzymes, such as lanosterol, lupeol, and cycloarthenol, dammarenediol, cucurbitadienol, and mixed-amyrin synthase (Fig. 3).

3.3. Expression of TpbAS in various organs of T. paniculatum

RT-PCR was performed to analyze the expression of *TpbAS* in different organs (leaf, stem, and root) of *T. paniculatum*. The housekeeping gene *NADH* was used as an internal standard to evaluate and equate the RNA integrity. The *TpbAS* transcript was detected in all organs with the highest expression in leaves, followed by stems and roots (Fig. S2).

3.4. Saponin production in various organs of T. paniculatum

Measurement of saponin content from different organs of *T. paniculatum* indicated that this compound was present at high levels in the leaves, whiles it was expressed at low levels in roots and stems (Fig. 4).

4. Discussion

In this study, a new gene from *T. paniculatum* with a high similarity with β-amyrin synthase from *V. hispanica* was identified. These two plants belong to the Portulacaceae family; thus, they may have the same or similar products with similar structures due to the corresponding biosynthesis enzymes [37].

The predicted molecular mass of protein encoded by this gene is similar to the molecular mass of oxidosqualene cyclase reported from other plants [31]. The QW motifs discovered repetitively in the deduced amino acids are also present in each oxidosqulene

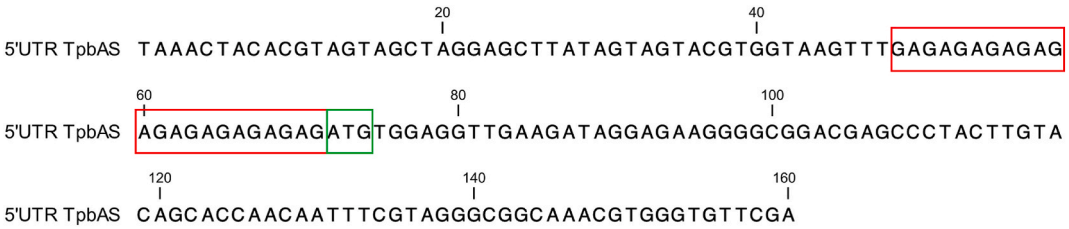

Fig. 1. The nucleotide sequence upstream of the ATG (Green box = Start codon) is the 5'-untranslated region (5'UTR). The (GA)n sequences (red box) were found in the 5'-untranslated region of *TpbAS*. The figure showed only the partial sequence of *TpbAS*. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

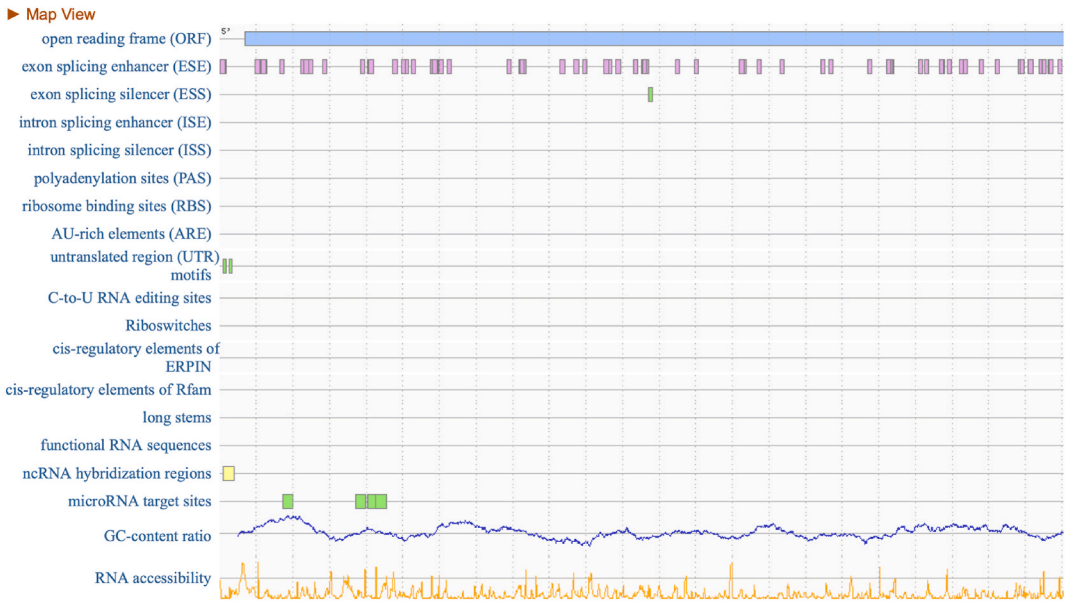

Fig. 2. RNA analysis result derived from integrated webserver RegRNA 2.0. The blue box indicating the ORF within the sequence; the purple box indicating the location of ESE within the sequence; the green box indicating the 5′-UTR region; the yellow box indicating ncRNA hybrization regions (The more information couldn't be provide since the sequence is encoding protein); the following green box indicating the site than can be targeted by miRNA. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

cyclases. This sequence is critical for protein structure and stability and its catalytic activity. The aromatic amino acids in this QW motif provides a negative charge that interacts with the cation intermediates formed in the cyclization reaction [13]. The aspartic acid in the DCTAE motif provides protons that attack the epoxide ring to trigger a successive polycyclization reaction. A mutation in this amino acid leads to a loss of enzymatic activity. Several aromatic amino acids are also found in this enzyme. These electron-rich amino acids including F474, F728, Y259, and W257 play a role in stabilizing of carbocation intermediates formed in the polycyclization reaction [51]. However, in the amino acid sequence of *TpbAS*, F474 lies at 475 and F728 lies at 730. This shift may have no implication as long as it is aligned as indicated in the alignment result and probably have no impact on protein folding. The tryptophan in the WCYCR motif plays a role in the stabilization of the oleanyl cation. Mutation of this tryptophan into leucine in β-amyrin synthase from *P. ginseng* leads to the major lupeol product in yeast expressing this mutated gene. Further mutation of the tyrosine that is conserved in all OSCs into histidine produces a dammarene-type saponin precursor [25]. The conserved motifs found in this predicted TpbAS protein and the phylogenetic tree result reinforce the argument that the family of oxidosqualene cyclases catalyzes the formation of sterols in fungi (*Saccharomyces cerevisiae*) and triterpenoid saponins is probably derived from the same ancestor [31]. The proteins tend to be clustered within the same oxidosqualene cyclases or the same genus. Further study must be conducted to confirm the activity of *TpbAS* using eukaryotic organisms such as *Saccharomyces cerevisiae* or *Pichia pastoris*.

Simple sequence repeats (SSRs) or microsatellites polypurine-polypyrimidine sequences GA/CT repeats distributed non randomly in 5′UTR, as (CT)n sequence presented in the tryptophan decarboxylase gene of *Catharanthus roseus*, could affect gene expression levels (Kumar and Bhatia, 2016). The (GA)n sequence found in the *TpbAS* 5′-UTR may have a similar role in controlling gene expression. Functional RNA motifs and sites analysis indicated exon splicing enhancers (ESE) and several microRNA target site candidates in the coding sequence that previously discovered in human. Some of the nucleotide sequences identified have purine-rich, containing GAR (GAG/GAA) motifs as indicated in the first discovered exon splicing enhancers (ESE) and many other motifs to be found later (Pertea et al., 2007). Analysis using RegRNA demonstrated the presence of ESE sequences recognized by Serine-/arginine-rich (SR) proteins previously found in viruses and animals. SR proteins involved in splicing are highly conserved in all metazoans and plants. The SR proteins in plants are more diverse compared with the animal kingdom performing more special function [52]. These elements may regulate nearby intron splicing in *TpbAS* to produce functional mRNA. The miRNA target site candidates could play a role in the modulation of this gene expression. A non-conserved miRNA that is weakly expressed and occurs temporally reported could regulate metabolite biosynthesis in plants [53]. In addition, previous research showed a miRNA namely miR163 targeted the gene involved in secondary metabolites biosynthesis in *Arabidopsis* sp [54]. More specific study needed to prove this prediction since those exon splicing enhancers (ESE) and microRNA target site candidates were identified in another kingdom based on the analysis and data provided by RegRNA 2.0. The recent study investigated the potential miRNA-based therapeutics to prevent and treat various diseases since the biogenesis and mechanism of plant and animal mRNAs in modulating gene expression showed less discrimination [55].

Analysis of in β-amyrin synthase gene expression in *C. asiatica* and *A. sedifolius* indicated the same result with the highest expression in leaves but undetectable in roots [39,41]. Various stressors are known to induce the production of secondary metabolites. Northern blot analysis showed the enhancement of β-amyrin synthase expression in soybean leaves after wounding and/or under

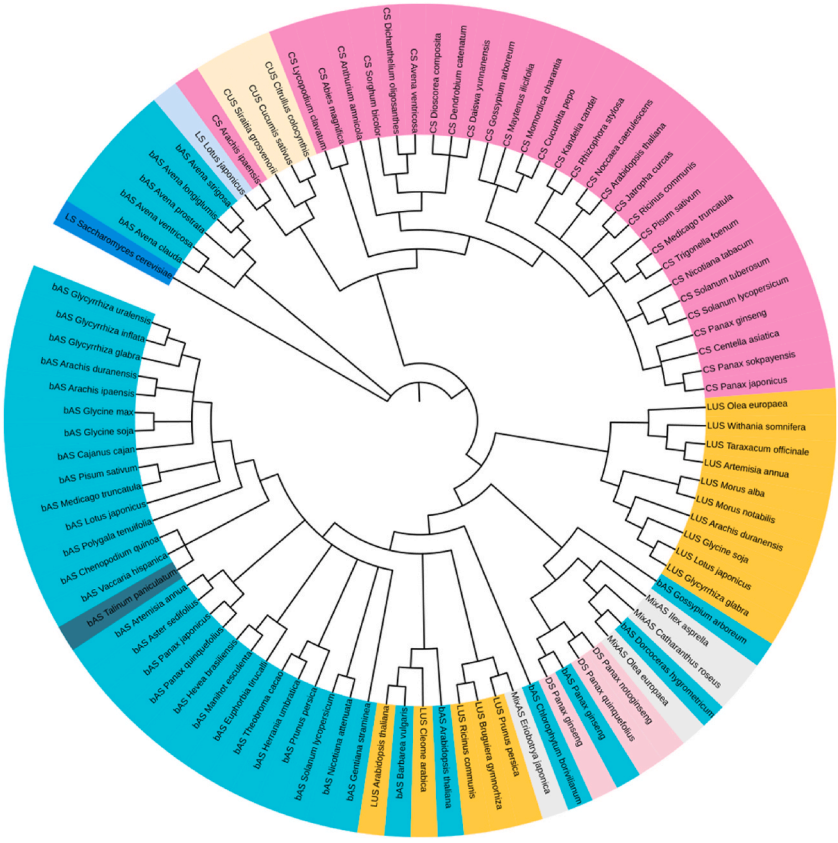

Fig. 3. Phylogenetic tree constructed by the neighbor-joining method using MEGA X. (bAS: β-amyrin synthase; CS: cycloarthenol synthase; LUS: lupeol synthase; LS: lanosterol synthase; TS: thalianol synthase; DS: dammarenediol synthase; MixAS: mixed-amyrin synthase; CUS: cucurbitadienol synthase). The amino acid sequences and their GenBank accession number are presented in Supplementary Table 3. The tosca green area represents bAS from plants other than *Talinum paniculatum*, the dark tosca green area represents bAS protein in this study, the pink area represents CS proteins, the light pink area represents DS proteins, the yellow area represents LUS proteins, the cream area represents CUS proteins, the grey area represents MixAS proteins, the light blue area represents LS protein, and the dark blue area represents the outgroup used in phylogenetic construction. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

low-temperature conditions [40]. Availability of nutrients could also modulate the expression of this gene as reported in *G. glabra* [56].

Taken together, the analysis of *TpbAS* expression and saponin content in various organs of *T. paniculatum* indicated that the synthesis and accumulation of saponin in this plant occur in the same organs. Plants can synthesize and accumulate saponins in various organs, depending on the utilized defense system. In some plants, saponins function in innate immunity, such as phytoanticipin, as they are produced constitutively without any triggering factors [57]. Certain stress conditions, such as seasonal changes and other abiotic stressors including light, and temperature can alter the production and composition of saponins [58]. The accumulation of secondary metabolites in root and stem herbs is mainly affected by growth periods, growing seasons, and growth years while the leaf age, harvesting season, and growth stage governed the accumulation in leaves [59]. For instance, phytochemical analysis using HPLC and gene expression analysis using transcriptomics approach identified that the leaves of *Paris polyphylla* accumulate more saponins during the vegetative stage. This consistent with the expression of most biosynthetic genes which are in abundance in leaves during the vegetative stage. During the growth process, the abundance of saponin in plant organs can change. In the fruiting stage, the expression of biosynthetic genes increases in the rhizomes and results in higher saponin concentration than other organs [60]. In addition, the saponins content of *Panax notoginseng* flower buds grown in different area and duration were varied. The following study indicated that the flower buds contain higher saponins than stems, leaves, and roots with a different type [61].

Organ cultures, including those of shoots, roots, and hairy roots have been developed to produce secondary metabolites in bioreactors [62]. The previous study used *in vitro* culture of various explants, such as buds, callus, cell suspension, and roots to produce triterpenoid saponins from *Primula veris* plants [63]. This in-depth optimization was performed to obtain a desirable culture condition with robust saponin production. In addition, methyl jasmonate treatment was administered to induce gene expression within the mevalonate pathway resulted in increased saponin production [64]. These synthesis and accumulation analyses indicate that organ type must be considered for optimizing saponin production and for minimizing the experimental process. Furthermore, the metabolic engineering approaches that induce the expression of single or multiple of the key enzymes that control the flux of desired metabolites can be performed to boost the accumulation of the desired bioproduct [65]. The homologous and heterologous expression of β-amyrin

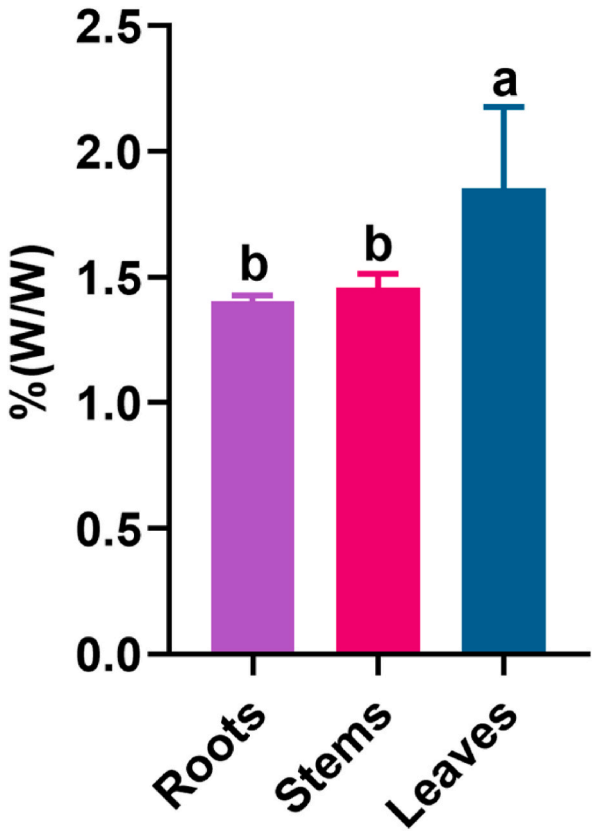

Fig. 4. Accumulation of saponin in various organs of *T. paniculatum*.

synthase could enhance the accumulation of oleanane-type triterpenoid saponin to meets the human needs.

5. Conclusion

The gene encoding β-amyrin synthase from *T. paniculatum* was successfully isolated and characterized. *TpbAS* consists of 2298 bp that encode 765 amino acid residues with (GA)n sequences in the 5′-UTR. Further functional RNA motifs and sites analysis predicted the present of exon splicing enhancers and silencer, as well as miRNA target sites candidate within *TpbAS* sequence. Protein analysis predicted QW, DCTAE, and WCYCR motifs that are conserved in oxidosqualene cyclase family. In addition, this research identified that the synthesis and accumulation of saponin in *T. paniculatum* occur at the same site.

Author contribution statement

Ika Qurrotul Afifah: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Indra Wibowo: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ahmad Faizal: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17707.

References

[1] C. Thanamool, P. Papirom, S. Chanlun, S. Kupittayanant, *Talinum paniculatum* (Jacq.) Gertn.: a medicinal plant with potential estrogenic activity in ovariectomized rats, Int. J. Pharm. Pharmaceut. Sci. 5 (2013) 478–485.
[2] S. Tolouei, G.N. da Silva, T.Z. Curi, M.T. Passoni, D. Ribeiro, H.G. Meldola, N. Grechi, G.S. Hey, R. Souza, A.C. Dos Santos, O.C. Beltrame, P.R. Dalsenter, A. J. Martino-Andrade, A. Gasparotto Junior, Effects of *Talinum paniculatum* (Jacq.) Gaertn. leaf extract on general toxicity and pubertal development of rats, Hum. Exp. Toxicol. 40 (2021) 124–135.
[3] C. Souto, B.R. Lorençone, A.A.M. Marques, R.A.C. Palozi, P.V.M. Romão, L.P. Guarnier, C.A.S. Tirloni, A.C. Dos Santos, R.I.C. Souza, P. Zago, F. Lívero, E.L. B. Lourenço, D.B. Silva, A. Gasparotto Junior, Cardioprotective effects of *Talinum paniculatum* (Jacq.) Gaertn. in doxorubicin-induced cardiotoxicity in hypertensive rats, J. Ethnopharmacol. 281 (2021), 114568.
[4] F. Menezes, T.A. Ishizawa, L.R.F. Souto, T.F. Oliveira, *Talinum paniculatum* (Jacq.) Gaertn. leaves - source of nutrients, antioxidant and antibacterial potentials, Acta Sci. Pol. Technol. Aliment. 20 (2021) 253–263.
[5] L.F. Dos Reis, C.D. Cerdeira, B.F. De Paula, J.J. Silva, L.F. Coelho, M.A. Silva, V.B. Marques, J.K. Chavasco, G. Alves-Da-Silva, Chemical characterization and evaluation of antibacterial, antifungal, antimycobacterial, and cytotoxic activities of *Talinum paniculatum*, Rev. Inst. Med. Trop. Sao Paulo 57 (2015) 397–405.
[6] M. Ramos, G. Silva, L.P. Duarte, V. Peres, R.R.S. Miranda, G.H.B. De Souza, V.J. Belinelo, S.A.V. Filho, Antinociceptive and edematogenic activity and chemical constituents of *Talinum paniculatum* Willd, J. Chem. Pharmaceut. Res. 2 (2010) 265–274.
[7] Y.S.W. Manuhara, A.N. Kristanti, E.S.W. Utami, Optimization of culture conditions of *Talinum paniculatum* Gaertn. adventitious roots in balloon type bubble bioreactor using aeration rate and initial inoculum density, Asian J. Bio. Sci. 8 (2015) 83–92.
[8] A. Faizal, A.V. Sari, Enhancement of saponin accumulation in adventitious root culture of Javanese ginseng (*Talinum paniculatum* Gaertn.) through methyl jasmonate and salicylic acid elicitation, Afr. J. Biotechnol. 18 (2019) 130–135.
[9] N.N. Erin, A. Yachya, A.N. Kristanti, D. Sugiarso, Y.S.W. Manuhara, Effect of carbon source variations on growth, physiological stress, and saponin levels of *Talinum paniculatum*, J. Trop. Biodivers. Biotechnol. 7 (2022), 69359.
[10] C.Y. Yang, J. Wang, Y. Zhao, L. Shen, X. Jiang, Z.G. Xie, N. Liang, L. Zhang, Z.H. Chen, Anti-diabetic effects of *Panax notoginseng* saponins and its major anti-hyperglycemic components, J. Ethnopharmacol. 130 (2010) 231–236.
[11] L. Feng, X.M. Liu, F.R. Cao, L.S. Wang, Y.X. Chen, R.L. Pan, Y.H. Liao, Q. Wang, Q. Chang, Anti-stress effects of ginseng total saponins on hindlimb-unloaded rats assessed by a metabolomics study, J. Ethnopharmacol. 188 (2016) 39–47.
[12] Q. Zhou, D.H. Yu, N. Zhang, S.M. Liu, Anti-inflammatory effect of total saponin fraction from *Dioscorea nipponica* Makino on gouty arthritis and its influence on NALP3 inflammasome, Chin. J. Integr. Med. 25 (2019) 663–670.
[13] G.M. Vieira Júnior, C.Q. da Rocha, T. de Souza Rodrigues, C.A. Hiruma-Lima, W. Vilegas, New steroidal saponin and antiulcer activity from *Solanum paniculatum* L, Food Chem. 186 (2015) 160–167.
[14] A.S. Mohamed, S.A. Mahmoud, A.M. Soliman, S.R. Fahmy, Antitumor activity of saponin isolated from the sea cucumber, *Holothuria arenicola* against ehrlich ascites carcinoma cells in swiss albino mice, Nat. Prod. Res. 35 (2021) 1928–1932.
[15] M.O. Nafiu, A.I. Adewuyi, T.A. Abdulsalam, A.O.T. Ashafa, Antimalarial activity and biochemical effects of saponin-rich extract of *Dianthus basuticus* Burtt Davy in *Plasmodium berghei*-infected mice, Adv. Tradit. Med. 22 (2022) 519–529.
[16] J.-L. Hu, S.-P. Nie, D.-F. Huang, C. Li, M.-Y. Xie, Extraction of saponin from *Camellia oleifera* cake and evaluation of its antioxidant activity, Int. J. Food Sci. Technol. 47 (2012) 1676–1687.
[17] M.H. Lee, J.H. Jeong, J.W. Seo, C.G. Shin, Y.S. Kim, J.G. In, D.C. Yang, J.S. Yi, Y.E. Choi, Enhanced triterpene and phytosterol biosynthesis in *Panax ginseng* overexpressing squalene synthase gene, Plant Cell Physiol. 45 (2004) 976–984.
[18] L. Peng, C. Hu, C. Zhang, Y. Lu, S. Man, L. Ma, Anti-cancer activity of *Conyza blinii* saponin against cervical carcinoma through MAPK/TGF-β/Nrf2 signaling pathways, J. Ethnopharmacol. 251 (2020), 112503.
[19] F. de Costa, A.C. Yendo, S.P. Cibulski, J.D. Fleck, P.M. Roehe, F.R. Spilki, G. Gosmann, A.G. Fett-Neto, Alternative inactivated poliovirus vaccines adjuvanted with *Quillaja brasiliensis* or Quil-a saponins are equally effective in inducing specific immune responses, PLoS One 9 (2014), e105374.
[20] A. Parveen, M.A. Farooq, W.W. Kyunn, A new oleanane type saponin from the aerial parts of *Nigella sativa* with anti-oxidant and anti-diabetic potential, Molecules 25 (2020) 2171.
[21] R. Fuentes, A. Ruiz-de-Angulo, N. Sacristán, C.D. Navo, G. Jiménez-Osés, J. Anguita, A. Fernández-Tejada, Replacing the rhamnose-xylose moiety of QS-21 with simpler terminal disaccharide units attenuates adjuvant activity in truncated saponin variants, Chemistry 27 (2021) 4731–4737.
[22] C.T.M. Kayukawa, M.A.S. Oliveira, E. Kaspchak, H.B.S. Sanchuki, L. Igarashi-Mafra, M.R. Mafra, Quillaja bark saponin effects on *Kluyveromyces lactis* β-galactosidase activity and structure, Food Chem. 303 (2020), 125388.
[23] A. Krivoruchko, J. Nielsen, Production of natural products through metabolic engineering of *Saccharomyces cerevisiae*, Curr. Opin. Biotechnol. 35 (2015) 7–15.
[24] J.Y. Han, G. J, Y.S. Kwon, Y.E. Choi, Regulation of ginsenoside and phytosterol biosynthesis by RNA interferences of squalene epoxidase gene in *Panax ginseng*, Phytochemistry 71 (2010) 36–46.
[25] T. Kushiro, M. Shibuya, Y. Ebizuka, β-Amyrin synthase - cloning of oxidosqualene cyclase that catalyzes the formation of the most popular triterpene among higher plants, Eur. J. Biochem. 256 (1998) 238–244.
[26] P.P. Shu, L.X. Li, Q.M. He, J. Pan, X.L. Li, M. Zhu, Y. Yang, Y. Qu, Identification and quantification of oleanane triterpenoid saponins and potential analgesic and anti-inflammatory activities from the roots and rhizomes of *Panax stipuleanatus*, J. Ginseng Res. 45 (2021) 305–315.
[27] Z.W. Wu, W.B. Li, J. Zhou, X. Liu, L. Wang, B. Chen, M.K. Wang, L. Ji, W.C. Hu, F. Li, Oleanane- and ursane-type triterpene saponins from *Centella asiatica* exhibit neuroprotective Effects, J. Agric. Food Chem. 68 (2020) 6977–6986.
[28] H.J. Park, H. Kwon, J.H. Lee, E. Cho, Y.C. Lee, M. Moon, M. Jun, D.H. Kim, J.W. Jung, β-Amyrin ameliorates alzheimer's disease-like aberrant synaptic plasticity in the mouse hippocampus, Biomol. Ther. (Seoul) 28 (2020) 74–82.
[29] M.S. Kwun, H.J. Lee, D.G. Lee, β-amyrin-induced apoptosis in *Candida albicans* triggered by calcium, Fungal Biol 125 (2021) 630–636.
[30] S. Yalçın, S. Yalçınkaya, F. Ercan, In silico detection of inhibitor potential of *Passiflora* compounds against SARS-Cov-2(Covid-19) main protease by using molecular docking and dynamic analyses, J. Mol. Struct. 1240 (2021), 130556.
[31] K. Haralampidis, M. Trojanowska, A.E. Osbourn, Biosynthesis of triterpenoid saponin in plants, Adv. Biochem. Eng. Biot. 75 (2002) 31–49.

[32] M. Confalonieri, M. Cammareri, E. Biazzi, P. Pecchia, M.P.S. Fevereiro, A. Balestrazzi, A. Tava, C. Conicella, Enhanced triterpene saponin biosynthesis and root nodulation in transgenic barrel medic (*Medicago truncatula* Gaertn.) expressing a novel β-amyrin synthase (*AsOXA1*) gene, Plant Biotechnol. J. 7 (2009) 172–182.
[33] M. Takemura, R. Tanaka, N. Misawa, Pathway engineering for the production of β-amyrin and cycloartenol in *Escherichia coli*-a method to biosynthesize plant-derived triterpene skeletons in *E. coli*, Appl. Microbiol. Biotechnol. 101 (2017) 6615–6625.
[34] D. Li, Y. Wu, P. Wei, X. Gao, M. Li, C. Zhang, Z. Zhou, W. Lu, Metabolic engineering of *Yarrowia lipolytica* for heterologous oleanolic acid production, Chem. Eng. Sci. 218 (2020), 115529.
[35] Z. Huang, J. Lin, Z. Cheng, M. Xu, X. Huang, Z. Yang, J. Zheng, Production of dammarane-type sapogenins in rice by expressing the dammarenediol-II synthase gene from *Panax ginseng* C, A. Mey, Plant Sci. 239 (2015) 106–114.
[36] H. Kohda, Y. Yamaoka, S. Morinaga, M. Ishaki, M. Darise, Saponins from *Talinum triangulare*, Chem. Pharm. Bull. 40 (1992) 2557–2558.
[37] J.P. Vincken, L. Heng, A. de Groot, H. Gruppen, Saponins, classification and occurrence in the plant kingdom, Phytochemistry 68 (2007) 275–297.
[38] I. Iturbe-Ormaetxe, K. Haralampidis, K. Papadopoulou, A.E. Osbourn, Molecular cloning and characterization of triterpene synthases from *Medicago truncatula* and *Lotus japonicus*, Plant Mol. Biol. 51 (2003) 731–743.
[39] O.T. Kim, M.Y. Kim, S.M. Huh, D.G. Bai, J.C. Ahn, B. Hwang, Cloning of a cDNA probably encoding oxidosqualene cyclase associated with asiaticoside biosynthesis from *Centella asiatica* (L.) Urban, Plant Cell Rep. 24 (2005) 304–311.
[40] E. Chung, C.W. Cho, K.Y. Kim, J. Chung, J.I. Kim, Y.S. Chung, K. Fukui, J.H. Lee, Molecular characterization of the GmAMS1 gene encoding β-amyrin synthase in soybean plants, Russ. J. Plant Physiol. 54 (2007) 518–523.
[41] M. Cammareri, M.F. Consiglio, P. Pecchia, G. Corea, V. Lanzotti, J.I. Ibeas, A. Tava, C. Conicella, Molecular characterization of β-amyrin synthase from *Aster sedifolius* L. and triterpenoid saponin analysis, Plant Sci. 175 (2008) 255–261.
[42] Y.L. Liu, Y.F. Cai, Z.J. Zhao, J.F. Wang, J. Li, W. Xin, G.M. Xia, F.N. Xiang, Cloning and functional analysis of a β-amyrin synthase gene associated with oleanolic acid biosynthesis in *Gentiana straminea* MAXIM, Biol. Pharm. Bull. 32 (2009) 818–824.
[43] T. Moses, J. Pollier, A. Faizal, S. Apers, L. Pieters, J.M. Thevelein, D. Geelen, A. Goossens, Unraveling the triterpenoid saponin biosynthesis of the African shrub *Maesa lanceolata*, Mol. Plant 8 (2015) 122–135.
[44] J.C. Li, C. Wang, W.T. Qi, C.L. Liu, Cloning and functional characterization of the β-amyrin synthase gene from *Bupleurum chinense*, Biol. Plant. (Prague) 64 (2020) 314–319.
[45] J. Yin, J. Yang, H. Ma, T. Liang, Y. Li, J. Xiao, H. Tian, Z. Xu, Y. Zhan, Expression characteristics and function of CAS and a new beta-amyrin synthase in triterpenoid synthesis in birch (*Betula platyphylla* Suk.), Plant Sci. 294 (2020), 110433.
[46] T. Murashige, F. Skoog, A revised medium for rapid growth and bio assays with tobacco tissue cultures, Physiol. Plantarum 15 (1962) 473–497.
[47] T.H. Chang, H.Y. Huang, J.B. Hsu, S.L. Weng, J.T. Horng, H.D. Huang, An enhanced computational platform for investigating the roles of regulatory RNA and for identifying functional RNA motifs, BMC Bioinf. 14 (Suppl 2) (2013) S4.
[48] S. Kumar, S. Bhatia, A polymorphic (GA/CT)n- SSR influences promoter activity of Tryptophan decarboxylase gene in *Catharanthus roseus* L. Don, Sci. Rep. 6 (2016), 33280.
[49] J. Fiallos-Jurado, J. Pollier, T. Moses, P. Arendt, N. Barriga-Medina, E. Morillo, V. Arahana, M. de Lourdes Torres, A. Goossens, A. Leon-Reyes, Saponin determination, expression analysis and functional characterization of saponin biosynthetic genes in *Chenopodium quinoa* leaves, Plant Sci. 250 (2016) 188–197.
[50] I.Q. Afifah, Isolation and characterization of gene encoding β-amyrin synthase involved in saponin biosynthesis in *Talinum paniculatum* (Jacq.) Gaertn, Master's Thesis. Institut Teknologi Bandung (2018).
[51] T. Hoshino, β-Amyrin biosynthesis: catalytic mechanism and substrate recognition, Org. Biomol. Chem. 15 (2017) 2869–2891.
[52] K. Kumar, S.K. Sinha, U. Maity, P.B. Kirti, K.R.R. Kumar, Insights into Established and Emerging Roles of SR Protein Family in Plants and Animals, Wiley Interdiscip. Rev. RNA, 2022, e1763.
[53] D. Liu, R. Mewalal, R. Hu, G.A. Tuskan, X. Yang, New technologies accelerate the exploration of non-coding RNAs in horticultural plants, Hortic. Res. 4 (2017), 17031.
[54] D.W. Ng, C. Zhang, M. Miller, G. Palmer, M. Whiteley, D. Tholl, Z.J. Chen, cis- and trans-Regulation of miR163 and target genes confers natural variation of secondary metabolites in two Arabidopsis species and their allopolyploids, Plant Cell 23 (2011) 1729–1740.
[55] A.N. Saiyed, A.R. Vasavada, S.R.K. Johar, Recent trends in miRNA therapeutics and the application of plant miRNA for prevention and treatment of human diseases, Futur. J. Pharm. Sci. 8 (2022) 24.
[56] H. Hayashi, P.Y. Huang, A. Kirakosyan, K. Inoue, N. Hiraoka, Y. Ikeshiro, T. Kushiro, M. Shibuya, Y. Ebizuka, Cloning and characterization of a cDNA encoding β-amyrin synthase involved in glycyrrhizin and soyasaponin biosyntheses in licorice, Biol. Pharm. Bull. 24 (2001) 912–916.
[57] A. Faizal, D. Geelen, Saponins and their role in biological processes in plants, Phytochemistry Rev. 12 (2013) 877–893.
[58] A. Szakiel, C. Paczkowski, M. Henry, Influence of environmental abiotic factors on the content of saponins in plants, Phytochemistry Rev. 10 (2011) 471–491.
[59] Y. Li, D. Kong, Y. Fu, M.R. Sussman, H. Wu, The effect of developmental and environmental factors on secondary metabolites in medicinal plants, Plant Physiol. Biochem. 148 (2020) 80–89.
[60] X. Gao, X. Zhang, W. Chen, J. Li, W. Yang, X. Zhang, S. Li, C. Liu, Transcriptome analysis of *Paris polyphylla* var. yunnanensis illuminates the biosynthesis and accumulation of steroidal saponins in rhizomes and leaves, Phytochemistry 178 (2020), 112460.
[61] Z. Wang, Y.-y. Chen, H.-j. Pan, L. Wei, Y.-h. Wang, C.-h. Zeng, Saponin accumulation in flower buds of *Panax notoginseng*, Chin. Herb. Med. 7 (2015) 179–184.
[62] F. Bourgaud, A. Gravot, S. Milesi, E. Gontier, Production of plant secondary metabolites: a historical perspective, Plant Sci. 161 (2001) 839–851.
[63] V. Okršlar, I. Plaper, M. Kovač, A. Erjavec, T. Obermajer, A. Rebec, M. Ravnikar, J. Žel, saponins in tissue culture of *Primula veris* L, in vitro cell, Dev. Biol.-Plant 43 (2007) 644–651.
[64] S. Rahimi, Y.J. Kim, D.C. Yang, Production of ginseng saponins: elicitation strategy and signal transductions, Appl. Microbiol. Biotechnol. 99 (2015) 6987–6996.
[65] X. Lu, K. Tang, P. Li, Plant metabolic engineering strategies for the production of pharmaceutical terpenoids, Front. Plant Sci. 7 (2016) 1647.