Spermatophyte Sesquiterpene Synthases

Subjects: Cell & Tissue Engineering

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Sesquiterpenes are important defense and signal molecules for plants to adapt to the environment, cope with stress, and communicate with the outside world, and their evolutionary history is closely related to physiological functions. In this study, the information of plant sesquiterpene synthases (STSs) with identified functions were collected and sorted to form a dataset containing about 500 members. The phylogeny of spermatophyte functional STSs was constructed based on the structural comparative analysis to reveal the sequence–structure–function relationships. We propose the evolutionary history of plant sesquiterpene skeletons, from chain structure to small rings, followed by large rings for the first time and put forward a more detailed function-driven hypothesis. Then, the evolutionary origins and history of spermatophyte STSs are also discussed. In addition, three newly identified STSs CaSTS2, CaSTS3, and CaSTS4 were analyzed in this functional evolutionary system, and their germacrene D products were consistent with the functional prediction. This demonstrates an application of the structure-based phylogeny in predicting STS function.

spermatophyte sesquiterpene synthetase sesquiterpene phylogenetic analysis functional evolution

1. Introduction

Terpenes constitute a large class of chemically and structurally diverse natural products in the plant kingdom and serve multiple physiological and ecological functions. At present, more than 25,000 terpenoid structures and 80,000 compounds have been found [1]. Sesquiterpenes are the most complex group with structural diversity, and more than 300 kinds of basic skeletons have been found, which are widely distributed in plants and microorganisms [2]. Sesquiterpenes play important roles in interactions with pollinators and seed dispersers [3], direct defenses against herbivores [4] and pathogens [5], mediate plant–plant and plant–microbe interactions [6], and help acclimation to biotic and abiotic environmental stress [7].

The main structure of sesquiterpene is biosynthesized by STSs, which convert farnesyl diphosphate (FPP) into the sesquiterpene skeleton [8]. Common to all STSs is the formation of highly reactive carbocationic intermediates which can undergo a great variety of rearrangements resulting in a huge number of different sesquiterpene structures [9]. It is initiated by the divalent metal ion-dependent ionization of the substrate FPP, and the following cyclization reactions depending on which carbon−carbon double bond reacts with the initially formed allylic carbocation (**Figure 1**) [10]. One type involves cyclization of farnesyl cation to yield (*E,E*)-germacradienyl cation (C10-C1 closure) or (*E*)-humulyl cation (C11-C1 closure) rings. The other type is initiated by isomerization of the

C2–C3 double bond of farnesyl cation to the tertiary nerolidyl cation. Then, the cisoid conformer of nerolidyl cation can undergo cyclization to either the central or distal double bond forming bisabolyl cation (C6-C1 closure), cycloheptanyl cation (C7-C1 closure), (Z,E)-germacradienyl cation (C10-C1 closure), or (Z)-humulyl cation (C11-C1 closure). The resulting cationic intermediate undergoes deprotonation or addition of water before termination sesquiterpene [1].

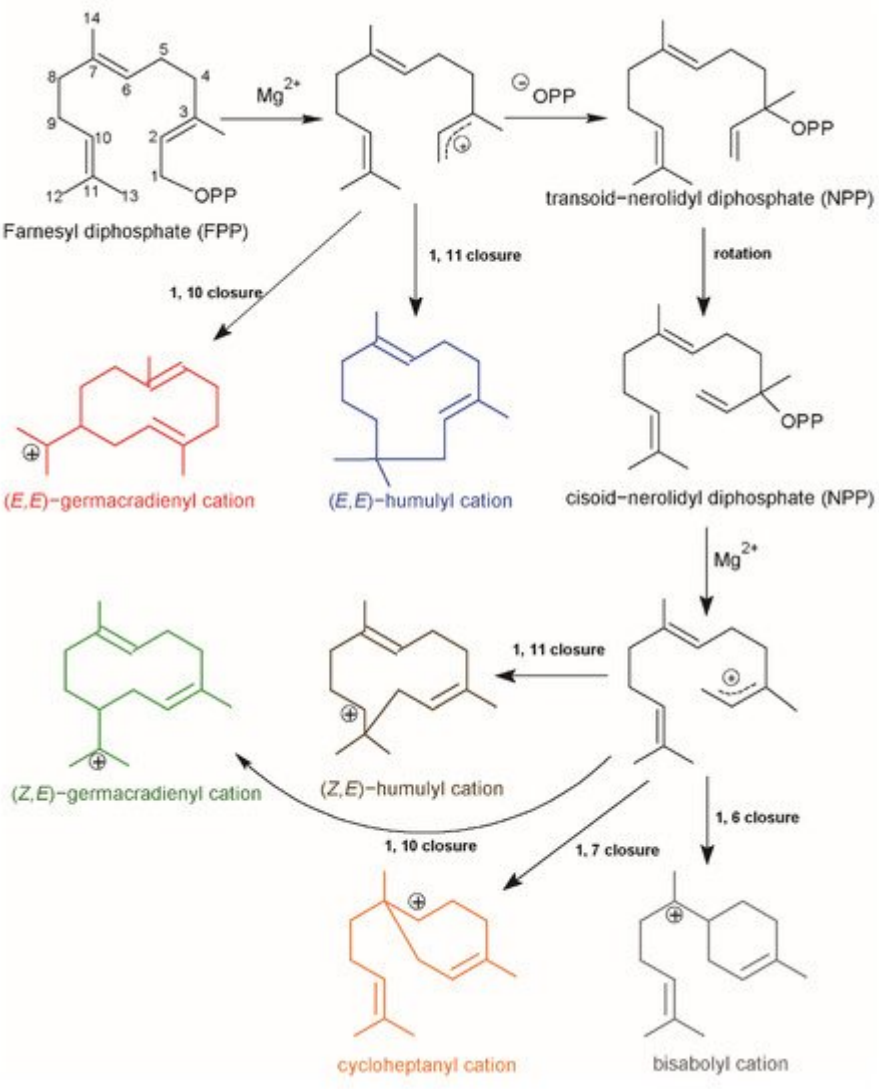

Figure 1. The reaction mechanism of sesquiterpene production starting with FPP. Farnesyl cation is formatted by losing the diphosphate moiety (OPP). Then, the farnesyl cation can be converted to the nerolidyl cation. Possible cyclizations for both cations are indicated in the figure.

The ancestral bifunctional diterpene synthases having γβα tri-domain architecture, presumably catalyzing production of gibberellin intermediate ent-kaurene, seem to the ancestor of all plant terpene synthases (TPS) [11]. The ancient gene duplication and sub-functionalization led to separate class II diterpene cyclases (γβα tri-domain) and subsequently class I TPSs (βα didomain) including the STSs [12]. At present, most studies on STS evolution focused on the phylogenetic location of STSs [13][14]. The TPS family is classified into seven subfamilies designated as TPS-a, TPS-b, TPS-c, TPS-d, TPS-e/f, TPS-g, and TPS-h [11]. Among these, the STSs are predominantly

distributed in the angiosperm-specific TPS-a clade and the gymnosperm-specific TPS-d clade [12], and there are few STSs in the TPS-b, TPS-g, and TPS-e/f subfamilies [11]. However, functional evolution of plant STSs has not yet been clarified. It is difficult to predict enzyme function from STS sequences, because STSs represent a very diverse set of enzymes with a wide range of sequence similarities. Janani et al. gathered 262 plant STS sequences with experimentally characterized products, hoping to choose likely functional residues for mutagenesis studies; unfortunately, they did not reveal any general rules for STS function prediction [15].

2. Current Insights

Along with the species evolution, plants have evolved to produce a different collection of terpenenes to accommodate their biotic and abiotic environment [16]. The plant sesquiterpenes gradually formed and might be a potential result of escalating defense and counter-defense between plants and specialized herbivores [17][18]. Exploring the evolutionary origin and history of STSs can not only help to understand the evolutionary pattern and reaction mechanism, but also preliminarily predict the function of STSs. At present, there are many phylogenetic analyses of plant TPSs, most of which focus on taxonomic studies to indicate the evolutionary behavior of TPSs among and within species [19][20], without functional selection of sequences. In this study, all 394 spermatophyte STSs were divided into five distinct groups according to structure-based phylogenetic analysis to explore the evolutionary patterns of plant STSs and sesquiterpenes.

Many genes are involved in sesquiterpene biosynthesis in the genome of each plant species, which also provides a large platform for the evolution of new sesquiterpenes via gene duplication and subfunctionalization [21][22]. Intron loss, mutations, and coevolution with natural enemies are considered to be the most important evolutionary dynamics of STSs. Evolution in STSs is often the result of intron loss or mutations that lead to subfunctionalization or function loss [23]. For example, a large fragment loss of δ-selinene synthase 2 (*Agsel2*) from *A. grandis* was found around intron X that led to *Agsel2* being transcribed as a pseudogene [24]. Single amino acid W279A switch converts δ-cadinene synthase (CAD1-A) into germacradien-4-ol synthase [25]. New sesquiterpenes keep arising in specific plant lineages, potentially as an outcome of coevolution with natural enemies [17]. Different plant lineages have evolved the ability to make additional “specialized” metabolites that are implicated in defense or the attraction of beneficial organisms, which indicates a dominant process dynamic evolution in STSs to the chemical diversity in plants.

STSs have various evolutionary forms. It can also be expected that STSs with altered subcellular localization and new substrate specificities would have evolved. Although TPSs often have broad substrate specificity and accept GPP, FPP, or GGPP in vitro, their function may be narrower in planta due to their subcellular localization [26]. Monoterpene synthases and diterpene synthases typically contain N-terminus signal peptides and are transported into plastids, STSs, however, are usually found in the cytosol [9]. There is increasing evidence for an exchange of TPS subcellular localization, especially under stress conditions [27][28]. Examples include the *AmNES/LIS-1/2* and *CsLIS/NES-1/2* analyzed above in the TPS-g subfamily. In this scenario, driven by adaptive evolution, ancestral monoterpene synthases losing the N-terminus signal peptide changed their substrate pool and gradually evolved into STSs. Models for gymnosperm TPS evolution proposed that STSs evolved from diterpene synthases through

loss of introns, which resulted in, among other changes, the complete loss of the γ domain [24]. Based on this model, *Abies grandis* a-bisabolene synthase *Ag1* (C6-C1 closure), a three-domain plant STSs, is potentially an intermediate in the evolutionary history from diterpene to sesquiterpene synthase [29].

Distinct catalytic features of the STSs arose early in spermatophyte evolution and the reactions have become more complex over time. In the evolution of STSs, it is easier to form acyclic sesquiterpenes than cyclic sesquiterpenes according to phylogenetic analysis. Acyclic sesquiterpenes were formed directly from farnesyl cation or nerolidyl cation by proton loss or addition of water [9]. For cyclic sesquiterpenes, they can be formed by typically catalyzing reaction cascades with additional steps, such as the isomerization of carbon–carbon double bond in the initial cation to allow alternate ring closures or additional cyclization [9]. Successive gene duplications and the subsequent accumulation of mutations led to the multitude of STSs, many of which catalyze more complex reactions than the ancestor. Although it is universally accepted that evolution of natural product biosynthesis has led to the formation of more and more complex structures, this process has rarely been documented at the level of a specific enzyme and plant group [30]. Overall, we speculated the early possible evolutionary process of spermatophyte STSs is from acyclic sesquiterpenes to cyclic sesquiterpenes, and the C6-C1 closure sesquiterpenes (small rings) may have formed earlier than C10-C1/C11-C1 closure sesquiterpenes. Interestingly, in some specific STSs, evolution may stop at a certain stage to form a series of characteristic metabolites under certain selective pressures. For example, *Artemisia* species' STSs are clustered in the A1 clade of the TPS-a subfamily (**Figure 2**) and obviously originated from a common ancestor dedicated to producing 1,6-cyclized sesquiterpenes. Among these, AaADS from *Artemisia annua* produces the artemisinin specific intermediate amorpha-4,11-diene (C6-C1 cyclized bicyclic sesquiterpenes) [31]; however, other STSs highly homologous to ADS from *Artemisia* species cyclize FPP to (+)-a-bisabolol (C6-C1 monocyclic sesquiterpenes) [32].

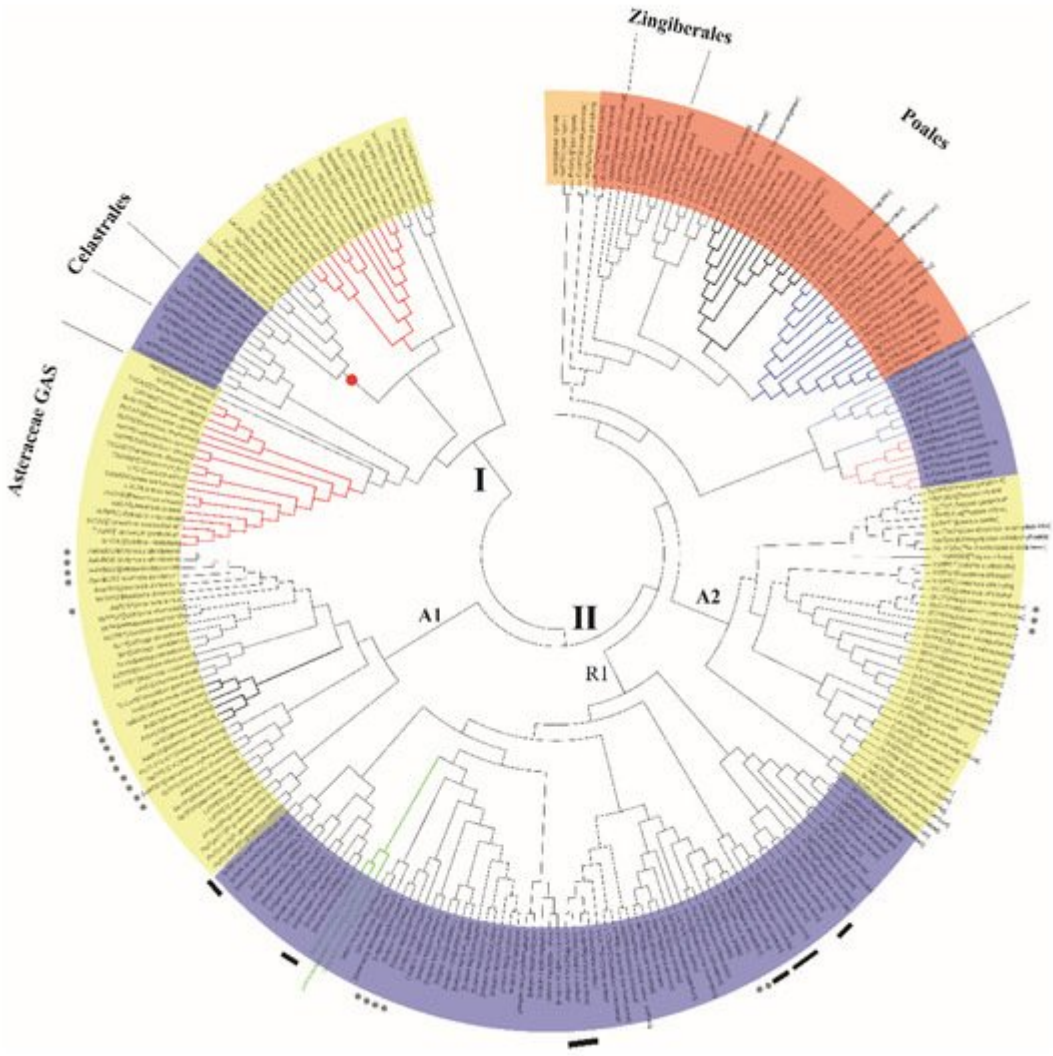

Figure 2. The phylogeny of spermatophyte STSs in the TPS-a subfamily. Blue shadow represents the *Rosanae* STSs, yellow shadow represents the *Asterids* STSs, red shadow represents the *monocot* STSs, and orange shadow represents the *magnoliid* STSs. Red branches show the STSs with identical products, blue branches show the STSs with 1,11-cyclized functions and green branches show the probable HGT members from *Santalum album*. STSs producing 1,6-cyclized sesquiterpenes are marked with grey dots, and the neighbor STSs producing 1,10- or 1,11-cyclized products, respectively, are marked with black rectangles.

Systematic study on the evolutionary changes of STS structure is an effective way to elucidate its function. Over the last three decades, high-resolution crystal structures have become available for STSs, and the enzymatic structure–function relationships have revealed the evolutionary relationships of STSs [1][12]. It was recognized early that the structure of STS products depending on the initial substrate conformation imposed by the enzymatic active site cavity [33]. For example, *SaSQS2* is the representative of C6-C1 cyclized STSs, and the shape of FPP conformation is close to natural straight chain in its active site cavity [34]. For C10-C1 cyclized STSs of *TEAS* [35] and *XC1* [36], the shapes of FPP are obviously curved. For the formation of acyclic sesquiterpene, we choose the medium/long-chain-length prenyl pyrophosphate synthase as the representative because there is no crystal structure of acyclic STSs, in which the FPP conformation is almost natural straight chain [37] (Figure S2). Overall, in

the evolution of STSs, the change of residues in the active site cavity made the straight chain FPP gradually bend, which made the C1 carbocation gradually approach the intramolecular double bond, and endowed STSs with the ability to form small ring and even large ring sesquiterpenes.

Horizontal gene transfer (HGT) also plays an important role in the evolution of STSs [38]. Sixteen *Santalum* STSs have been functionally charactered (Table S3), among which, 13 STSs are clustered in TPS-b and their products are mainly C6-C1 cyclized sesquiterpene β-bisabolene (**Figure 3**b), while three STSs are clustered in the R1 clade of the TPS-a subfamily and their products are mainly C10-C1 cyclized sesquiterpenes germacrene D-4-ol, hedycaryol and C11-C1 cyclized sesquiterpene a-humulene (**Figure 2**). This indicates that the evolutionary sources of STSs in these two parts are completely different. Sandalwood is a semi-parasitic plant, whose survival is inseparable from the host plant. Some studies have shown that sandalwood prefers to parasitize on nitrogen fixing woody plants [39], and HGT events between sandalwood and host plants have also been reported [40][41], which may indicate that STSs in sandalwood come from HGT. These parasitic plants are likely to obtain the synthesis ability of terpenenes and other secondary metabolites from the host through HGT, so as to better adapt to the environment or communicate with the host.

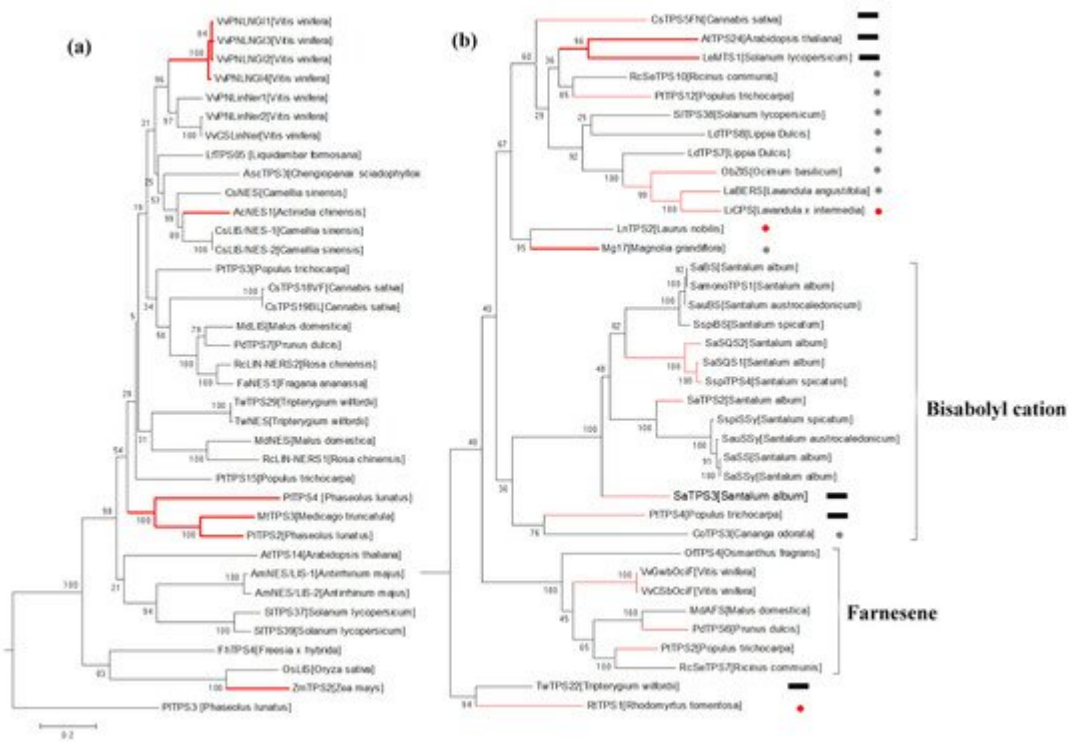

Figure 3. (**a**) The phylogeny of spermatophyte STSs in the TPS-g subfamily. STSs with diterpene synthases function in vitro are shown by red branches and uniformly distributed in the phylogenetic tree. (**b**) The phylogeny of spermatophyte STSs in the TPS-b subfamily. STSs with monoterpene synthases function in vitro are shown by red branches. STSs producing 1,6-cyclized sesquiterpenes and 1,10/1,11-cyclized sesquiterpenes are marked with grey dots and red dots, respectively. Products of STSs marked with black rectangles are acyclic farnesene or nerolidol.

In this comprehensive analysis, on the one hand, we collected the information of plant STSs with identified functions and constructed the phylogeny of plant functional STSs based on the structural comparative analysis, to reveal the sequence–structure–function relationships. On the other hand, we highlighted our incomplete understanding of the evolutionary pattern of sesquiterpenes in spermatophytes (**Figure 4**), from chain structure to small rings, followed by large rings for the first time, and discussed the evolutionary origins and history of STSs from spermatophyte plants. Then, we proved our evolutionary pattern is useful in predicting the function of STSs by three germacrene D synthases.

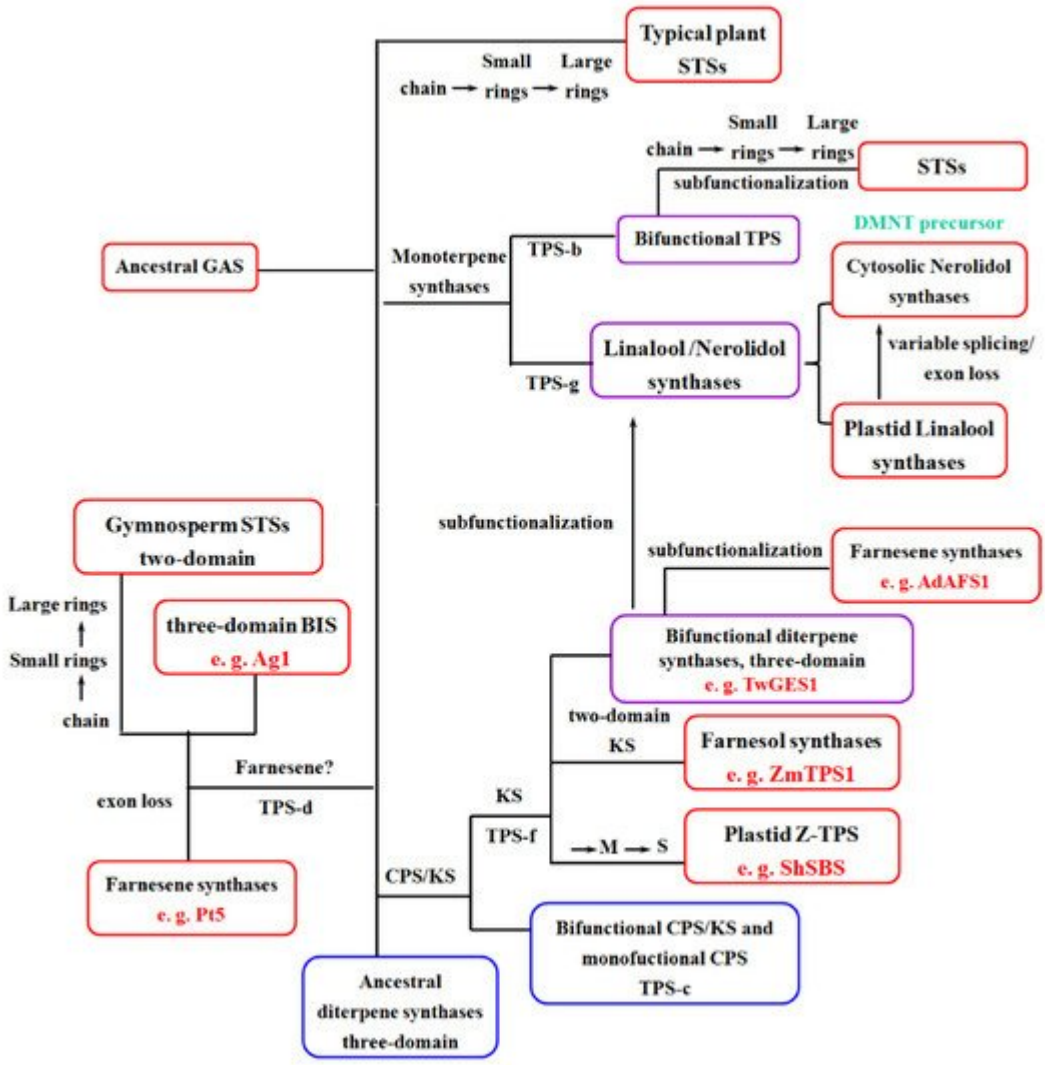

Figure 4. The proposed evolutionary history of spermatophyte STSs. Blue boxes represent ancestral TPSs, red boxes represent STSs, and purple boxes represent transition TPSs. The character “M” represents monoterpene synthases and the character “S” represents STSs. The red words represent the examples of STSs, the green words represent functional products of STSs.

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