Journal of Plant Physiology 221 (2018) 144–155

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Non-plastidial expression of a synthetic insect geranyl pyrophosphate synthase effectively increases tobacco plant biomass

Gui Li[a,b], Jing Xi[a], Xiaoming Ji[a], Ming-Zhuo Li[a], De-Yu Xie[a,*]

[a] Department of Plant & Microbial Biology, North Carolina State University, Raleigh, NC, 27695, United States
[b] State Key Laboratory of Tree Genetics and Breeding, Research Institute of Forestry, Chinese Academy of Forestry, Key Laboratory of Tree Breeding and Cultivation of the State Forestry Administration, Beijing, 100091, China

ARTICLE INFO

Keywords:
Synthetic geranyl pyrophosphate synthase
Plant synthetic biology
Plant biomass
Metabolic engineering

ABSTRACT

Designing effective synthetic genes of interest is a fundamental step in plant synthetic biology for biomass. Geranyl pyrophosphate (diphosphate) synthase (GPPS) catalyzes a bottleneck step toward terpenoid metabolism. We previously designed and synthesized a plant (*Arabidopsis thaliana*)-insect (*Myzus persicae*, Mp) GPPS-human influenza hemagglutinin (HA) cDNA, namely *PTP-MpGPPS-HA* (or *PTP-sMpGPPS-HA*, s: synthetic), to localize the protein in plastids and improve plant biomass. To better understand the effects of different subcellular localizations on plant performance, herein we report *PTP-sMpGPPS-HA* re-design to synthesize a new *MpGPPS-HA* cDNA, namely *sMpGPPS-HA*, to express a non-plastidial sMpGPPS-HA protein. The *sMpGPPS-HA* cDNA driven by a 2 × S 35S promoter was introduced into *Nicotiana tabacum* Xanthi. *PTP-MpGPPS-HA* and PMDC84 vector transgenic plants were also generated as positive and negative controls, respectively. Eighteen to twenty transgenic T0 lines were generated for each *sMpGPPS-HA, PTP-sMpGPPS-HA*, and PMDC84. Transcriptional genotyping analysis demonstrated the expression of *sMpGPPS-HA* in transgenic plants. Confocal microscopy analysis of transgenic progeny demonstrated the non-plastidial localization of sMpGPPS-HA. Growth of T1 transgenic and wild-type control plants showed that the expression of *sMpGPPS-HA* effectively increased plant height by 50–80%, leaf numbers and sizes, and dry biomass by 60–80%. Calculation of the vegetative growth rates showed that the expression of *sMpGPPS-HA* increased plant height each week. Moreover, *sMpGPPS-HA* expression promoted early flowering and reduced leaf carotenoid levels. In conclusion, non-plastidial expression of the novel sMpGPPS-HA was effective for improving tobacco growth and biomass. Our data indicate that research examining different subcellular localizations facilitates a better understanding of *in planta* functions of proteins encoded by synthetic cDNAs.

1. Introduction

Numerous recent review articles have concluded that a renewable and sustainable biomass resource is the primary factor for the production of sufficient biofuel (Ben-Iwo et al., 2016; Kircher, 2015; Lee et al., 2015; Williams et al., 2016; Xie et al., 2014). Unfortunately, a large number of investigations has revealed the lack of a single natural resource or crop system that can help solve the biomass limitations for biofuel production (Kircher, 2015; Kosinkova et al., 2015; Voloshin et al., 2016; Williams et al., 2016). The cost of using natural resources for feedstock also remains to be improved in terms of economic effectiveness (Williams et al., 2016). To overcome these limitations, different countries or organizations have been strategically endeavoring to investigate and identify appropriate natural renewable systems (Andrianov et al., 2010; Ben-Iwo et al., 2016; Kosinkova et al., 2015; Testa et al., 2014; Vanhercke et al., 2014; Voloshin et al., 2016; Williams et al., 2016; Xie et al., 2014). Accordingly, a few plants have been identified as prominent territorial renewable biomass resources, such as sorghum, switch grass, sugar cane, poplar tree, and willow tree (Tazoe et al., 2016; Williams et al., 2016). In addition, a few algae, particularly marine algae, have been identified as aquatic sustainable natural resources for biofuel production (Kosinkova et al., 2015; Lee et al., 2015; Voloshin et al., 2016). Furthermore, both academic institutions and industries have been attempting to develop potent biotechnologies to create novel renewable resources for biofuel feedstocks. One primary effort is to identify genes of interest to create superior agronomic traits of plants for field or aquatic growth. To date, multiple plant genes have been tested to increase biomass in both model and biofuel crops. Acid phosphatases (APases) comprise a group of enzymes that hydrolyze the release of phosphorous (Pi) from Pi monoesters and

* Corresponding author.
E-mail address: dxie@ncsu.edu (D.-Y. Xie).

https://doi.org/10.1016/j.jplph.2017.12.014
Received 14 July 2017; Received in revised form 8 December 2017; Accepted 16 December 2017
Available online 20 December 2017
0176-1617/

diesters and, thus, are appropriate candidates to increase free Pi in soil for plant growth. Purple acid phosphatase (PAP) is the largest subgroup of APases. A PAP gene (*AtPAP18*) was cloned from *Arabidopsis thaliana*. When it was overexpressed in tobacco plants, the biomass of the transgenic plants was increased (Zamani et al., 2012). Gibberellins (gibberellic acids, GAs) are plant growth regulators that promote plant growth and flowering. A GA receptor gene was isolated from *Galega orientalis,* and its ectopic expression was demonstrated to increase the biomass of transgenic tobacco plants (Li et al., 2013). Anaphase-promoting complex (APC) is a type of multi-subunit E3 ligase that regulates cyclin-dependent kinases (CDKs) and cyclins (Cyc) to control cell division checkpoints. An *APC10* was cloned from *A. thaliana* and then introduced into tobacco plants (Lima et al., 2013). The overexpression of *APC10* and *AtCDC27* was shown to significantly increase transgenic tobacco growth (Lima et al., 2013). These promising reports demonstrate the significance of identifying genes of interest as a useful first step for biomass improvement.

Geranyl pyrophosphate synthase (GPPS, synonym: geranyl diphosphate synthase, GDS) ([EC 2.5.1.1.]) is a short-chain isopentyl diphosphate synthase. It catalyzes the formation of geranyl pyrophosphate through the condensation of dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP) (Fig. 1A). In animals, GPPS solely controls the formation of cholesterol and terpenoids. In plants, given that the biosynthesis of terpenoids is compartmentalized by the mevalonic acid (MVA) in the cytosol and 2-C-methyl-D-erithritol, 2,4-cyclopyrophosphate (MEP) pathway in the plastids, GPPS is the key enzyme in the biosynthesis of plant monoterpenoids, diterpenoids, GAs, chlorophylls, and other metabolites (Fig. 1A). In addition, recent studies have shown a potentially new role of a tomato (*Lycopersicon esculentum*) GPPS (LeGPPS), which was demonstrated to functionally involve plant growth (van Schie et al., 2007). A reduced expression level of this gene led to a dwarf phenotype, which was associated with a decrease in gibberellins.

Recently, we searched and designed genes of interests coupled with a synthetic approach for biomass improvement and plant terpenoid biosynthesis manipulation (Xi et al., 2016). For proof of concept, we used a *Myzus persicae GPPS* (*MpGPPS*) gene as a template to design a novel plant-insect-influenza cDNA. The MpGPPS has been reported to be a novel prenyltransferase that displays dual geranyl/farnesyl diphosphate synthase activity in the aphid *M. persicae* (Vandermoten et al., 2008). The open reading frame (ORF) of the synthetic cDNA was designed to consist of three cDNA fragments: an *A. thaliana* plastidial transit peptide (PTP) sequence, a truncated *MpGPPS* sequence, and a short human influenza hemagglutinin (HA) sequence, namely *PTP-MpGPPS-HA (or namely PTP-sMpGPPS-HA,* s: synthetic). We introduced this synthetic cDNA into a newly emerging biofuel crop, *Camelina sativa*, and targeted the enzyme to the plastids. The expression of *PTP-sMpGPPS-HA* led to enhancement of plant growth and an increase in leaf numbers (Xi et al., 2016). However, whether non-plastidial expression of sMpGPPS-HA can affect plant growth remains unknown. This knowledge will help fully understand the effects of synthetic genes on plant form and function. We hypothesize that a non-plastidial

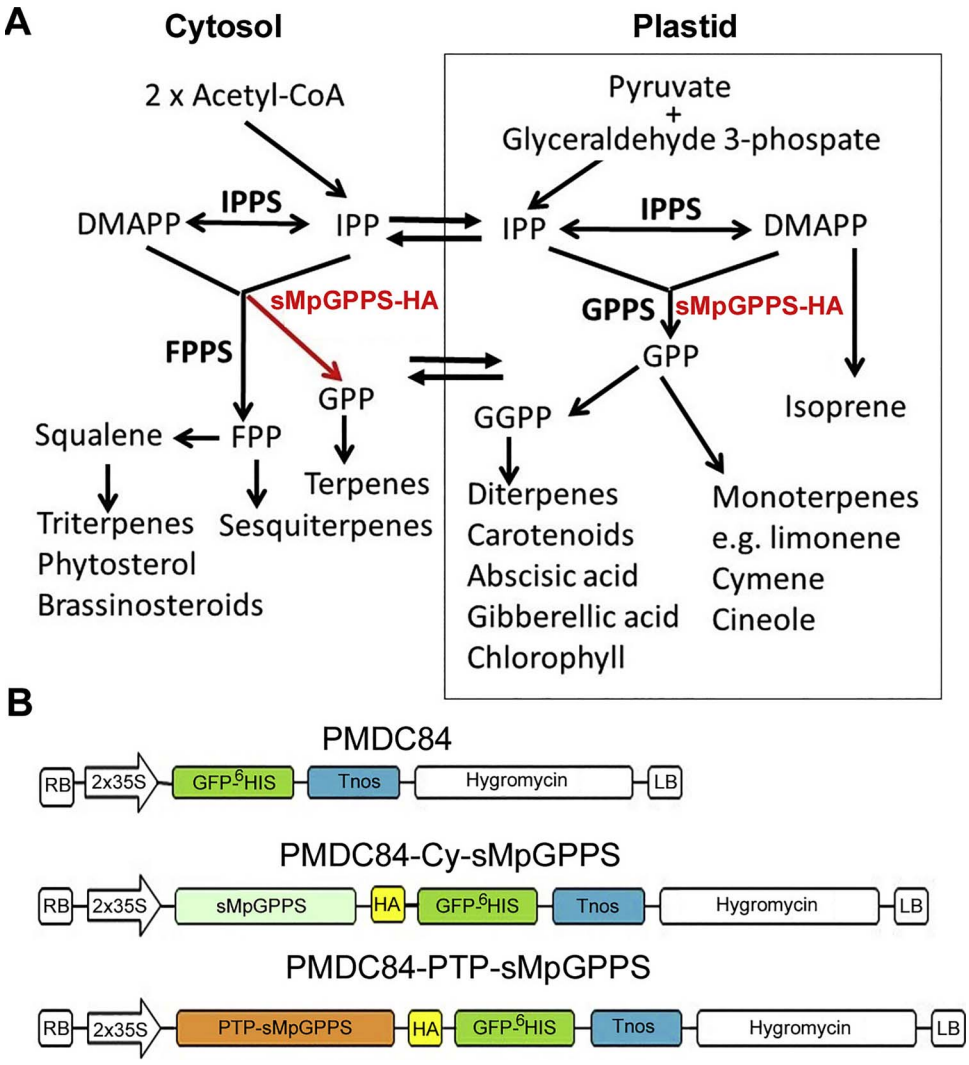

Fig. 1. A simplified schematic showing the localization of geranyl pyrophosphate synthase (GPPS) and T-DNA cassettes for expression of a synthetic geranyl pyrophosphate synthase (sMpGPPS) cDNA designed from *M. persicae* GPPS. A, The bottleneck step catalyzed by GPPS in the formation of terpenoids and introduction of a sMpGPPS-HA into plants; B, T-DNA maps showing the expression of synthetic cDNAs, a PMDC84-Cy-sMpGPPS cassette expressing a novel *sMpGPPS-HA* cDNA for non-plastidial localization of protein, a PMDC84-Pl-sMpGPPS cassette expressing a *PTP-sMpGPPS-HA* cDNA for plastidial localization of protein as a positive control, and the binary vector PMDC84 showing the T-DNA cassette for hosting synthetic cDNAs and the vector control.