Heterogeneous expression of chloroplast-localized geranylgeranyl pyrophosphate synthase confers fast plant growth, early flowering and increased seed yield

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Abstract
Geranylgeranyl pyrophosphate synthase (GGPS) is a key enzyme for a structurally diverse class of isoprenoid biosynthetic metabolites including gibberellins, carotenoids, chlorophylls and rubber. We expressed a chloroplast-targeted GGPS isolated from sunflower (Helianthus annuus) under control of the cauliflower mosaic virus 35S promoter in tobacco (Nicotiana tabacum). The resulting transgenic tobacco plants expressing heterogeneous GGPS showed remarkably enhanced growth (an increase in shoot and root biomass and height), early flowering, increased number of seed pods and greater seed yield compared with that of GUS-transgenic lines (control) or wild-type plants. The gibberellin levels in HaGGPS-transgenic plants were higher than those in control plants, indicating that the observed phenotype may result from increased gibberellin content. However, in HaGGPS-transformant tobacco plants, we did not observe the phenotypic defects such as reduced chlorophyll content and greater petiole and stalk length, which were previously reported for transgenic plants expressing gibberellin biosynthetic genes. Fast plant growth was also observed in HaGGPS-expressing Arabidopsis and dandelion plants. The results of this study suggest that GGPS expression in crop plants may yield desirable agronomic traits, including enhanced growth of shoots and roots, early flowering, greater numbers of seed pods and/or higher seed yield. This research has potential applications for fast production of plant biomass that provides commercially valuable biomaterials or bioenergy.

Introduction
Geranylgeranyl pyrophosphate synthase (GGPS) is an important branch-point enzyme for terpenoid biosynthesis. It catalyses the farnesyl pyrophosphate (FPP) branching reaction via condensation of FPP and isopentenyl pyrophosphate (IPP) to produce geranylgeranyl pyrophosphate (GGPP). GGPS plays different roles in different organisms. In plants, GGPP is an essential precursor for compounds that are required for plant growth and development. These include pigments such as carotenoids and chlorophylls (Okada et al., 2000), phytohormones such as gibberellins (GAs), abscisic acid, strigolactone (Thabet et al., 2012) and cytosolic products such as geranylgeranylated Rho, geranylgeranylated Rac and geranylgeranylated Rab G proteins (Brown and Goldstein, 1993).

Gibberellins regulate diverse plant growth processes such as germination, cell elongation, cell division and the development of flowers and fruits (Olszewski et al., 2002). Gibberellins are synthesized from the precursor, ent-kaurene. This precursor is produced from GGPP via a two-step cyclization. Carotenoids are synthesized from two molecules of GGPP by head-to-head condensation and play an essential role in protecting plants against potentially harmful photo-oxidative processes (Okada et al., 2000).

There have been many reports of the isolation and characterization of plant GGPS genes in Sinapis alba (Laferrière and Beyer, 1991), Capsicum annuum (Badillo et al., 1995; Kuntz et al., 1992), Coleus forskohlii (Engprasert et al., 2004), Lupinus albus (Aitken et al., 1995), Arabidopsis (Beck et al., 2013; Okada et al., 2000; Scolnik and Bartley, 1994; Zhu et al., 1997), Jatropha curcas (Lin et al., 2010) and Catharanthus roseus (Bantignies et al., 1995; Thabet et al., 2012). Our group previously cloned GGPS from Helianthus annuus and confirmed its functional activity by performing a complementation assay with the chromosomal crt gene cluster of carotenogenic bacteria (Oh et al., 2000).

Although many plant GGPS genes have been isolated and characterized, the effect of GGPS transgenes on endogenous GA content, plant growth and plant phenotype has not yet been examined. Metabolic engineering of different plants through the manipulation of the isoprenoid pathway would be of great commercial importance. In this report, we show that the sunflower GGPS protein with proven functional activity (Oh et al., 2000) is localized to chloroplasts, and heterologous expression of the sunflower gene (HaGGPS) in tobacco plants results in higher GA levels, rapid growth, early flowering, increased numbers of flowers and seed pods and higher seed yields compared with those of control plants. Similar phenotypes including rapid growth, early flowering and/or increased seed yield are observed in transgenic Arabidopsis thaliana and dandelion (Taraxacum brevicorniculatum) plants expressing HaGGPS.
Results
Selection of transgenic tobacco plants and analysis of transgene expression

For heterologous expression studies of a Helianthus annuus GGPS gene (NCBI accession number AF020041) in Nicotiana tabacum, we constructed the plant expression vectors pBI121-35S::HaGGPS (Figure 1a) and pBI121-35S::GUS as a control. The target gene was transferred into tobacco plants using Agrobacterium-mediated transformation. Transgenic tobacco plants were selected to have T2/T3 homozygous lines in the selection media. These plants were initially tested using PCR analysis to validate the integration of the HaGGPS gene into the genomic DNA of the transgenic tobacco plants. Real-time qPCR analyses confirmed the expression of the sunflower GGPS gene in transgenic tobacco leaves (Figure 1b). Next, GGPS enzymatic activity was determined in the transgenic tobacco plants. The activity of GGPS to synthesize GGPP from the substrates FPP and IPP was 69 pmol/h/mg protein, which was 4.4-fold higher than that in GUS-control plants (Figure 1c).

To examine the effects of HaGGPS expression on the growth and development of other plant species, we also generated transgenic Arabidopsis and dandelion (T. brevicorniculatum) plants expressing HaGGPS (Figure 1d,e). In the transgenic Arabidopsis plants, GGPS enzymatic activity was 1.8-fold higher than that in wild-type plants (Figure 1c). Interestingly, the expression levels of endogenous GGPS genes showed an up-regulated tendency except AtGGPS3 in HaGGPS-transgenic Arabidopsis (Figure S1). The tendency of up-regulation in endogenous plastid GGPS genes was consistent with the data obtained from the transcription profile analysis of WT and two HaGGPS-transgenic Arabidopsis lines (Table S4). Similar concurrent up-regulation of endogenous carotenoid biosynthesis genes except one was previously reported in transgenic tomato plants expressing a bacterial carotenoid gene (crtI) (Romer et al., 2000). AtGGPS11 with 70% sequence identity to HaGGPS was dominantly expressed among endogenous genes in 16–19 day-grown mature plants (Figure S1c and Table S4) but not in 10 day-grown young seedlings (Figure S1b).

Subcellular localization of Helianthus GGPS in transgenic tobacco

Twelve members of the GGPS family in Arabidopsis were reported to be localized to different subcellular compartments including chloroplast, endoplasmic reticulum (ER) or mitochondria (Beck et al., 2013). According to the WoLF PSORT programme (Horton et al., 2007), the sunflower GGPS protein was predicted to be localized in the plastid. To determine the subcellular localization of the HaGGPS protein, we performed transient expression assays of HaGGPS-GFP fusion proteins in tobacco plant leaves. The green dots in Figure 2a represent the fluorescence of HaGGPS-GFP fusion proteins, and the red spots in Figure 2b represent chlorophyll autofluorescence. On the basis of the bright-field image (Figure 2c), the fluorescence merged image in Figure 2d shows that the green dots of GFP signal mostly overlap with the red fluorescence of chloroplasts, which confirms that the sunflower GGPS is localized to chloroplasts.

![Figure 1](image-url)
Fast increase in shoot length and shoot biomass in transgenic tobacco plants

To investigate the effect of HaGGPS expression on the growth of the transgenic plants, we measured plant height in 60 days old tobacco plants of T3 transgenic lines. As shown in Figure 3a, the height of HaGGPS-transgenic lines was 2.6-fold taller compared with that of GUS-control plants. Consistent with these results, the length of the stem internodal cells showed a fast increase by 33% in HaGGPS-transgenic lines compared with that in GUS-control plants (Figure S2a). Similar enhancement in plant growth (2.2- to 2.7-fold taller than GUS-control or wild-type) was also observed in T1- and T2-transgenic tobacco lines (data not shown). To assess the effect of HaGGPS overexpression on biomass increase, we determined the fresh shoot weights of 60 days old tobacco plants. There was a significant (2.1-fold) enhancement in shoot biomass increase in the transgenic tobacco plants compared with control plants (Figure 3b). The growth curve assays at the different time intervals demonstrate that the accelerated growth is spread over the whole growth period, but plant height at the time of flowering was similar in HaGGPS-expressing and control lines (Figure S2b). The effect of HaGGPS expression on seed germination was also investigated. However, there was no significant difference in seed germination between the HaGGPS-expressing and control lines (Figure S3a), suggesting that growth enhancement by the HaGGPS transgene occurs primarily after germination.

Early flowering, more flowers/seed pods and higher seed yields in transgenic tobacco plants

T3 transgenic tobacco plants were grown in the greenhouse to measure the effect of HaGGPS expression on flowering time. Most of the GGPS-transgenic lines flowered 2 weeks earlier than control plants, which normally require 15 weeks to flower. Thus, HaGGPS expression reduces the flowering time by approximately 15% (Figure 3c,d).

We also examined the effect of HaGGPS expression on the number of flowers and seed pods. HaGGPS-transgenic tobacco lines produced greater numbers of flowers than the GUS-control lines (Figure 3e). The average number of seed pods per plant in HaGGPS-transgenic lines was 95 ± 2 compared with 55 ± 4 in control plants. The number of seed pods in HaGGPS-transgenic lines was 73% higher than that in control lines (Figure 3f). HaGGPS expression also showed an effect on seed yield. The seed yield in transgenic plants increased by about 50% compared with that in control plants (Table 1). The HaGGPS-transgenic plants had an approximately 15% increase in average seed weight compared with that in control plants (Figure S3b), which partially contributes to the observed increase in seed yield. This trait would be valuable if it could be introduced into other important food or oil crop species, in which seed yield is a critical factor for crop improvement.

HaGGPS expression in transgenic tobacco enhances root growth

A detailed analysis of root morphology was performed with homozygous plants of the T3 generation. The effect of HaGGPS expression on root growth and architecture was analysed initially on Murashige and Skoog (MS) medium to examine patterns of early growth. The elongation of primary roots in 7 days old HaGGPS-transgenic tobacco plants increased by 1.6-fold compared with that in GUS-control plants. The total root length in 15 days old plants increased by 1.6-fold in transgenic plants compared with that in control plants (Figure 4a,b). The formation of lateral and adventitious roots was significantly enhanced in HaGGPS-transgenic lines (Figure 4a). Transgenic plants were
cultivated in soil to test whether the observed increases in root length were maintained during subsequent plant development. Figure 4c shows that mature transgenic plants also possess a larger root system than that in control lines. At 60 days after germination, the fresh biomass of the root system of HaGGPS-transgenic plants was 3.0-fold higher than that of control plants (Figure 4d). These results suggest that HaGGPS expression in transgenic tobacco plants influences the growth of root tissues as well as shoot tissues.

HaGGPS expression alters gibberellin levels in transgenic tobacco lines

Phytohormones are involved in the growth and development of the plant. Many of the phenotypic characteristics of the HaGGPS-transgenic tobacco plants prompted us to determine whether they contained higher gibberellin levels than those in GUS-control plants. Therefore, we quantified the active gibberellins, GA1 and GA4, and their immediate precursors, GA20 and GA9, in HaGGPS-transgenic and GUS-control lines. Figure 5 shows the GA content in apical stems with leaves in 60 days old plants. In HaGGPS-transgenic tobacco lines, the contents of GA1 and its precursor GA20 were approximately twofold higher than those in control plants. The contents of GA4 and its immediate precursor GA9 in transgenic tobacco plants were also almost twofold higher than those in control plants.

To make clear the molecular basis for the GA content increase, we investigated whether GA biosynthesis genes were up-regulated in the HaGGPS-transgenic tobacco plants. The expression levels of ent-kaurenoic acid oxidase (NtKAO), which controls a rate-limiting step in GA biosynthesis (Regnault et al., 2014), were strongly up-regulated (28-fold) in the transgenic plants compared with those in control plants (Figure S4a). The expression levels of ent-kaurene synthase (NtKS) were up-regulated by 2.4-fold in transgenic plants compared with those in control plants (Figure S4b). Whereas, the levels of ent-kaurene oxidase (NtKO) and GA20-oxidase1 (NtGA20ox1) were not significantly modulated in the transgenic tobacco plants (Figure S4c,d). The results suggest that heterologous expression of HaGGPS in tobacco plants selectively up-regulates the expression of key GA biosynthesis genes, which is accompanied by an elevation of GA content levels. Transcription profile analysis of HaGGPS-transgenic Arabidopsis lines showed slightly different but similar results with significant up-regulation in the expression of AtKAO2 and AtKO1, but not of AtKAO1 (Table S4).

Table 1  Seed yields in transgenic tobacco plants

| Plant type       | Seed yield (g) |
|------------------|----------------|
| Control (GUS)    | 1.57 ± 0.23    |
| GGPS-1           | 2.29 ± 0.41*   |
| GGPS-2           | 2.40 ± 0.35*   |

Data are mean ± SD, n = 4, *P < 0.05.

Wild-type plants were examined as another control, and the results are described in the text.
Enhanced growth in HaGGPS-transgenic Arabidopsis and dandelion plants

Consistent with the growth observed in HaGGPS-transgenic tobacco plants, the 11 days old HaGGPS-transgenic Arabidopsis seedlings grown in MS media exhibited enhanced growth of root tissues compared with that in GUS-control seedlings (Figure 6a, b). More dramatic growth enhancement was observed in 18 days old HaGGPS-transgenic Arabidopsis plants that were transferred from MS media to soil and grown in pots for 7 days. These transgenic Arabidopsis plants showed a twofold increase in total fresh biomass and root length compared with those of control plants (Figure 6c, d). As observed in HaGGPS-transgenic tobacco plants (Figure 5b), accelerated growth occurred during the whole growth period of HaGGPS-transgenic Arabidopsis plants, but plant biomass at the time of flowering was similar in HaGGPS-transgenic and wild-type plants (Figure 5c). Only the flowering time was shortened by 2 days in HaGGPS-transgenic lines compared with that in wild-type plants (26 days). The seed yield and average seed weight increased by about 25% in HaGGPS-transgenic Arabidopsis lines compared with those of wild-type plants (Figure 5d).

HaGGPS-transgenic dandelion seedlings also displayed enhanced shoot and root growth compared with those in GUS-control plants (Figure 7a). The 6 days old HaGGPS-transgenic dandelion seedlings exhibited a threefold increase in root length compared with that of GUS-control plants (Figure 7b). We compared the general morphology of HaGGPS-transgenic dandelion plants with that of GUS-control plants after 30 days of growth in soil pots in a greenhouse (Figure 7c). Both shoot and root growth were strongly accelerated in HaGGPS-transgenic dandelion plants compared with that in GUS-control plants. The fresh biomass of HaGGPS-transgenic dandelion was twofold higher than that of control plants (Figure 7d).

HaGGPS-transgenic lines have normal morphology and chlorophyll content but reduced carotenoid content

According to our results, one of the possible reasons for the observed phenotype in HaGGPS-transgenic plant lines could be...
the increased GA content. However, the HaGGPS-transgenic lines had distinct phenotypes from those of the GA-biosynthetic gene-transgenic lines reported previously (Xiao et al., 2006). HaGGPS-transgenic tobacco plants did not exhibit an increase in petiole length (Figure 8a,b), or any significant changes in the sizes of leaf blades and seed pods (Figure S5a,b). Even though the expression levels of chlorophyll biosynthesis genes showed a down-regulated tendency in HaGGPS-transgenic tobacco lines (Figure S6), the chlorophyll contents were not decreased in the transgenic lines compared with control plants (Figure 8c,d). But the carotenoid content in HaGGPS-transgenic tobacco lines was reduced by 24% compared with that in control plants (Figure S7a). A similar reduction in carotenoid content (28%) was observed in HaGGPS-transgenic Arabidopsis lines compared with that in wild-type (Figure S7b). When we determined the expression levels of carotenoid biosynthesis genes, the expression of phytoene synthase (AtPSY), which is a potential key regulator of carotenoid biosynthesis (Ruiz-Sola and Rodriguez-Concepcion, 2012), was reduced by 34% in HaGGPS-transgenic Arabidopsis lines compared with that in control plants (Figure S7c). By contrast, the expression levels of tocopherol/ plastoquinone, strigolactone and ABA biosynthesis genes were not significantly modulated in the transgenic Arabidopsis plants (Figures S8–S10). These results suggest that heterologous expression of HaGGPS reduces the flux of isoprenoid metabolites to carotenoid biosynthesis but increases the flux of metabolites to GA biosynthesis by regulating the key genes involved.

Figure 6 Enhanced growth in T3 HaGGPS-transgenic Arabidopsis plants. (a and b) Photograph (a) and graph (b) showing accelerated seedling growth of HaGGPS-transgenic Arabidopsis plants compared with control GUS-transgenic plants on MS medium at 11 days after germination. (c and d) Photograph (c) and graph (d) showing enhanced growth of shoot and root tissues in HaGGPS-transgenic Arabidopsis plants compared with the control GUS-transgenic plants in soil pots at 18 days after germination. Error bars in (b and d) represent standard deviations obtained from three independent transgenic lines, \( n = 9 \) and \( 3 \), respectively, \( **P < 0.01 \).

Figure 7 Enhanced growth in T2 HaGGPS-transgenic dandelion plants. (a and b) Photograph (a) and graph (b) showing enhanced seedling growth of HaGGPS-transgenic dandelion plants compared with control GUS-transgenic dandelion plants on MS medium at 6 days after germination. (c and d) Photograph (c) and graph (d) showing accelerated increase in total fresh biomass of HaGGPS-transgenic dandelion plants compared with control GUS-transgenic plants in soil pots at 36 days after germination. Error bars in (b, d) represent standard deviations obtained from three independent transgenic lines, \( n = 3 \), \( *P < 0.05 \), \( **P < 0.01 \).
The CaMV 35S promoter showed a remarkable growth enhancement in plant height and biomass, early flowering and increased number of seed pods compared with GUS-control plants and wild-type plants throughout different generations.

The precursor for the plant growth hormone gibberellin is produced from GGPP. Gibberellins have a crucial role in cell elongation and division and development of flowers and fruits. Heterologous expression of HaGGPS appeared to enhance GA biosynthesis in tobacco plants. Biochemical analyses showed that there was indeed a twofold increase in GA contents in HaGGPS-overexpressing transgenic tobacco plants. Therefore, one of the possible reasons for the observed phenotype could be the increased GA content in the HaGGPS-transgenic tobacco lines. The role of GAs in stimulating plant cell elongation is well documented (Cowling and Harberd, 1999; Jones and Kaufman, 1983; Li et al., 2011). This is consistent with data showing a 33% increase in the length of the internodal cells in HaGGPS-transgenic tobacco lines compared with that in control plants. Our results also demonstrated that constitutive expression of HaGGPS promoted early flowering. Anthesis in the transgenic tobacco plants was approximately 20 days earlier than that in control plants, which require approximately 100 days of growth before anthesis. GA-dependent signalling was suggested to be part of the pathway that promotes flowering in Arabidopsis (Gocal et al., 2001). It was reported that floral homeotic genes are targets of gibberellin signalling in flower development (Yu et al., 2004). Therefore, these results suggest a positive correlation between early flowering and increased GGPS expression through GA-dependent signalling.

Although HaGGPS-expressing transgenic tobacco lines had higher levels of GA content, the observed phenotypes do not exactly match those previously reported for plants expressing GA-biosynthetic genes. Rather, HaGGPS-transgenic plants have unique phenotypes. It was reported that overexpression of GhGA20ox1 conferred GA overproduction characteristics to the transgenic tobacco plants, including elongation of petiole, stalk and hypocotyl. The transgenic plants also had reduced leaf blade and fruit sizes, fewer seeds per fruit and lighter green leaves (Xiao et al., 2006). Also, an extremely elongated-stem phenotype was observed in the transgenic tobacco plants expressing GA20-oxidase (Vidal et al., 2001). Such abnormally stem-elongated phenotype was not observed in HaGGPS-transgenic tobacco lines.
in our study. Overexpression of Carrizo citrange GA20ox1 in Carrizo citrange produced plants whose stems were much thinner (approximately half of the normal diameter) than those of control plants (Fagoga et al., 2007). Clearly, the abnormally and extremely stem-elongated phenotype and thinner stems are not good agricultural traits. The HaGGPS-transgenic tobacco lines did not exhibit an increase in petiole length, and reduced leaf blade and fruit sizes were not significant. These results indicate that HaGGPS expression does not have a serious negative effect on general plant morphology, in contrast to what has previously been reported for the transgenic plants expressing a GA-biosynthetic gene(s).

GGPS is a common precursor for many plastid-localized terpenoids, including the phyto side chain of chlorophyll. Therefore, overexpression of HaGGPS in transgenic tobacco could lead to the synthesis of chlorophyll and other terpenoid products. The transgenic tobacco HaGGPS lines had similar chlorophyll content to the GUS-control plants in spite of reduced expression of involved genes. The transient expression assay of HaGGPS-GFP fusion proteins used in this study demonstrated that the HaGGPS is localized to the chloroplast. Expression of HaGGPS in the chloroplast could promote a coordinated increase in the chlorophyll content concomitantly with an increase in shoot biomass. In this scenario, leaf colour would not change, and the chlorophyll content in HaGGPS-transgenic plants would be similar to that of GUS-control plants as observed in this study. By contrast, transgenic plants expressing a GA-biosynthetic gene have been reported to have a paler leaf colour and reduced chlorophyll content (Biemelt et al., 2004; Carrera et al., 2000; Huang et al., 1998). These results are understandable, because transgenic plants expressing a GA-biosynthetic gene primarily display an enhanced shoot growth without a proportional increase in the chlorophyll content. The morphological study of HaGGPS-transgenic plants revealed a fast increase in root length and biomass. This result has not been reported previously in studies of GA-biosynthetic gene overexpression. HaGGPS overexpression may also have effects on other phytohormones such as strigolactone and abscisic acid, which need to be elucidated in the future.

The transgenic HaGGPS lines show reduced carotenoid contents compared with those of control plants in both tobacco and Arabidopsis. Consistently, the expression levels of PSY gene, a potential key regulator of carotenoid biosynthesis (Ruiz-Sola and Rodriguez-Conception, 2012), were reduced by 34% in transgenic HaGGPS-expressing Arabidopsis lines. The expression levels of key GA biosynthesis genes were significantly higher in the HaGGPS-transgenic tobacco lines. These results indicate that GGPP produced in the plastid-localized HaGGPS-transgenic plants is utilized more for the gibberellin biosynthesis pathway than for the carotenoid biosynthesis pathway. By contrast, heterologous expression of the Gentiana GGPS in tobacco resulted in higher levels of \( \beta \)-carotene content (Li et al., 2009). Different from in the Arabidopsis genome (Beck et al., 2013), the identity of GGPS isoforms in the sunflower genome remains unknown.

To determine whether HaGGPS-induced enhancement of plant growth is limited to tobacco or whether it can be applied to other plant species, we tested Arabidopsis and dandelion. Consistent with HaGGPS-transgenic tobacco plants, both the HaGGPS-transgenic Arabidopsis and dandelion plants also showed significantly accelerated growth in both the shoot and root tissues compared with control plants.

Taken together, our studies indicate that the observed phenotype in HaGGPS-transgenic plants is not solely due to an increase in GA content, but is also related to the changes in other isoprenoid pathways such as carotenoid biosynthesis and the metabolic up- and down-modulations in several other cellular processes. However, the more detailed mechanisms underlying the observed phenotypes remain to be uncovered in future. As the HaGGPS-transgenic plants displayed fast growth, early flowering and higher yield of seeds, the development of GGPS-transgenic crops could be agronomically and industrially valuable. Further studies on the mechanisms and genetic manipulations based on these desirable agronomic traits might enhance the productivity of plant biomass and seeds that lead to the time-saving production of plant bioresources for biomaterials or bioenergy.

**Experimental procedures**

**Plant materials and data analysis**

Tobacco plants (Nicotiana tabacum cv. xanthi) were grown on MS-based medium with 3% (w/v) sucrose and 0.3% (w/v) phytotagel under 16 h of light with a light intensity of 20 \( \mu \text{mol}/m^2/s \) at 23 °C. Dandelion seeds (Taraxacum brevicorniculatum) were obtained from Dirk Prüfer at Muenster University, Germany, and were cultured as described previously (Tata et al., 2012). Arabidopsis thaliana plants were in the Colombia (Col-0) ecotype background and were grown on MS-based medium with 1% (w/v) sucrose. Eleven days after germination, plants were transferred to autoclaved soil in pots. Plant growth condition was a 16-h light and 8-h dark photoperiod (140 \( \mu \text{mol}/m^2/s \)) at 22 °C and 60% relative humidity. Data were analysed using the Student’s t-test.

**Construction of vector**

The GGPS gene was amplified from a Helianthus annuus cDNA library and cloned into Xba1 and BamHI sites of the pBI121 vector under the CaMV 35S promoter. The original pBI121 vector containing GUS (\( \beta \)-glucuronidase) was used as a control. Agrobacterium GV3101 transformants harbouring pBI121 plasmids with either GGPS or GUS were screened on solid YEP medium containing 50 mgL rifampicin and 50 mg/L kanamycin. The transformation of plasmids into Agrobacterium GV3101 was confirmed by PCR using HaGGPS-specific primers after plasmids were isolated from kanamycin-resistant GV3101 colonies.

**Generation of transgenic tobacco, dandelion and Arabidopsis lines carrying HaGGPS or GUS**

Transgenic tobacco and dandelion plants carrying p35S::HaGGPS or p35S::GUS were obtained via Agrobacterium-mediated transformation (Bae et al., 2005). Briefly, leaf explants were immersed in a suspension of Agrobacterium carrying binary vector for 20 min at 25 °C with gentle shaking. The explants were cocultured in the dark at 25 °C for 3 days on regeneration medium [MS salts, 3% sucrose, 1% glucose, 50 mg/L betaine, 0.2 mg/L 1-naphthaleneacetic acid, 2.0 mg/L benzylaminopurine (BA), 0.3% phytogel and 0.2 mm acetosyringone, pH 5.2] for tobacco plants and on a different regeneration medium [MS salts, 3% sucrose, 0.01 mg/L 3-indolebutyric acid (IBA), 2 mg/L BA, 0.3% phytogel, pH 5.8] for dandelion plants. After cocultivation, the explants were washed with cefotaxime (500 mg/L) to remove Agrobacterium. The explants were then transferred to each regeneration medium supplemented with kanamycin (100 mg/L) and cefotaxime (500 mg/L). After 3 weeks of culture in the dark, the tissues were maintained on the same medium for 4 weeks under light conditions. For rooting, explants with shoots were transferred to the medium.
containing 1/2 MS salts, 1.5% sucrose, 250 mg/L cefotaxime, 0.3% phytagel and 50 mg/L kanamycin at pH 5.8. After root formation, plants were transferred to soil for 3 weeks and then transferred to the greenhouse. Arabidopsis plants were transformed using the floral dip method (Clough and Bent, 1998) with Agrobacterium carrying p2BS::HaGGPS or p3BS::GUS. The independent line numbers obtained for tobacco, dandelion and Arabidopsis were 5, 3 and 3, respectively, and three independent lines showing the representative phenotypes were used for these experiments, respectively.

Real-time qPCR analyses
Total RNA was extracted from leaf tissues using TRizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. Then, 1 μg of total RNA was reverse transcribed using a cDNA synthesis kit (Intron Inc., Gyeonggi-do, Korea). Real-time qPCR was performed using SYBR green fluorescent dye (Bio Rad, Hercules, CA). The protocol was as follows: 95 °C for 5 min, 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, with a repetition of 38 cycles. Real-time qPCR was conducted using the gene-specific primers (Table S1). HaGGPS expression levels in transgenic plants were standardized by comparison with those of the reference genes (expression level = 1). The reference genes were ubiquitin (DQ138111) in tobacco plants, actin (JX425362) in dandelion plants and AT1G13320 (Hong et al., 2010) in Arabidopsis plants.

Quantification of gibberellins
Plants were harvested, immediately frozen in liquid nitrogen and stored at −70 °C. The method used for extraction and quantification of endogenous gibberellins was based on a previously established procedure (Lee et al., 1998). A 0.5 g lyophilized sample was used for GA analysis. Full-scan mode (the first trial), three major ions of the supplemented [2H2] GA internal standards (the second trial) and the endogenous gibberellins were monitored simultaneously. Standard GAs were purchased from Prof. Lewis Mander, Australian National University, Australia. The endogenous GA contents of GA1, GA3, GA9 and GA20 were calculated from the peak area ratios of 56/508, 284/286, 298/300 and 418/420, respectively. The data were calculated in nanograms per gram frozen dry weight, and the analysis was repeated three times.

Chlorophyll content measurement
Chlorophyll was extracted for 12 h from leaf discs (three discs with a diameter of 1.5 cm) placed in sealed vials with 10 mL of absolute methanol. Vials were continuously shaken in a covered water bath at ambient temperature. Extract aliquots of 100 μL were diluted to 1 mL with absolute methanol, and the absorbance was read at 645, 650, 663 and 665 nm. Total chlorophyll concentration was calculated (25.5 [A663] – 2.35 [A645]; Cb = 18.61 [A645] – 3.96 [A663]; and total carotenoids = 1000 [A652] – 2.270 x 645 + 81.4 x Cb)/227 (Biehler et al., 2010).

GGPS activity in plant tissues was determined according to a method described previously (Laskaris et al., 2000), which was based on the acid liability of the allylic diphosphates. Briefly, the incubation mixture was 100 μg of desalted protein extract, 50 mM Tris-HCl, pH 7.2, 10 μM leupeptin, 4 mM MnCl2, 2 mM MgCl2, 20% glycerol and 25 mM KF in a total volume of 200 mL. Before the addition of the substrates, the mixture was preincubated for 10 min in the presence of 10 μM iodoacetamide to inhibit the activity of IPP isomerase. The reaction was started by addition of [1-14C]-IPP (a final concentration of 4.86 μM, 55 μCi/ mmol) and FPP (a final concentration of 43.5 μM). After incubation for 30 min at 30 °C, the reaction was stopped by addition of 500 mL EtOH/HCl (1 : 1). Hydrolysis of the allylic diphosphates was allowed to proceed for 20 min at 37 °C and then 1.5 mL of toluene was added to extract the allylic alcohols. After extraction, 1 mL of the toluene layer was removed and mixed with 4 mL of OptiFluor (Packard, Waltham, MA), and the radioactivity incorporation was determined by liquid scintillation counting (Tri-Carb 4530; Packard). Blanks were performed with no protein extract and showed negligible incorporation of radioactivity.

Seed germination assay and cell length measurements
Seeds were surface sterilized and then placed on MS medium. Seed germination was observed under the microscope. Seed germination was considered on the day when the first radicle was observed. Internodes of 30 days old plants were chosen for vertical section, and the length of the epidermal cells was measured under a Nikon (Walpole, MA) Microphot-FXA microscope.

Illumina sequencing and bioinformatics analysis
Paired-end RNA sequencing with Illumina HiSeq2500 was performed with 16-day-old plant leaves of WT and two
HaGGPS-transgenic Arabidopsis lines. The data were refined to clean reads by Q ≥ 20 and length ≥25 before read mapping using DynamicTrim and LengthSort of SolexaQA softwares (Massey University, Palmerston North, New Zealand). Reads for each sequence tag were mapped to transcripts of Arabidopsis thaliana using Bowtie2 (mismatches ≤ 2). DEGs between WT and two GPPS-transgenic Arabidopsis lines were identified using DESeq (twofold change and FDR ≤ 0.01 of binomial test). Functional enrichment analysis was performed by DAVID programme. The up- and down-regulated DEGs were analysed with default criterion (counts ≥ 2 and EASE score ≤ 0.1) of each GO term.

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Conflict of interest

The authors have no conflict of interest to declare.

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Supporting information

Additional Supporting information may be found in the online version of this article:

Figure S1 Comparison of the expression levels of endogenous GGPS genes between wild-type and HaGGPS-transgenic Arabidopsis plants.

Figure S2 Stem internodal cell lengths and growth curve assays at different time intervals.

Figure S3 Comparison of seed germination, average seed weight and seed yield between control and HaGGPS-transgenic plants.

Figure S4 Comparison of the expression levels of GA biosynthesis genes between control and HaGGPS-transgenic tobacco plants.

Figure S5 Comparison of plant developmental phenotypes between control and HaGGPS-transgenic tobacco plants.

Figure S6 Comparison of chlorophyll biosynthesis gene expression levels between wild-type and HaGGPS-transgenic Arabidopsis lines.

Figure S7 Comparison of carotenoid content and carotenoid biosynthesis gene expression levels between GUS-control (or wild-type) and HaGGPS-transgenic plants.

Figure S8 Comparison of tocopherol and plastoquinone biosynthesis gene expression levels between wild-type and HaGGPS-transgenic Arabidopsis plants.

Figure S9 Comparison of strigolactone biosynthesis gene expression levels between wild-type and HaGGPS-transgenic Arabidopsis plants.

Figure S10 Comparison of ABA biosynthesis gene expression levels between wild-type and HaGGPS-transgenic Arabidopsis plants.

Table S1 Oligonucleotide primers used in the real-time qPCR analysis.

Table S2 Functional classification of the differentially expressed genes obtained from RNA sequencing in two HaGGPS-transgenic Arabidopsis lines relative to wild-type plants.

Table S3 List of the differentially expressed genes (up- or down-regulated) in both the two HaGGPS-transgenic Arabidopsis lines relative to wild-type plants (Presented separately in a excel file).

Table S4 Fold change in the expression levels of endogenous AtGGPS and gibberellin biosynthesis genes in two HaGGPS-transgenic Arabidopsis lines (GGPS-2 and GGPS-4) relative to wild-type plants (Presented separately in a excel file).