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ORIGINAL PAPER

DoDELLA1, a DELLA protein from *Dioscorea opposite*, regulates the growth and development in transgenic tobacco by controlling gibberellin level

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Abstract

Gibberellins (GAs) are involved in many aspects of plant growth and development. DELLA proteins are major repressors of GA signaling as a transcription factor to regulate plant growth. Yam (*Dioscorea opposite*) tuber development is regulated by GA. In a previous study, we have identified and characterized a gene encoding DELLA protein and named as *DoDELLA1* from yam tuber. However, its function is unclear. The results showed that *DoDELLA1* expression patterns of leaf, stem and tuber in two yam cultivars, Guihuai 16 (GH16) and Guihuai 5 (GH5), were in response to GA_3. DELLA and VHYNP domain of DoDELLA1 protein had transcriptional capacity, LH, VHIID and SAW domain were not involved in transcriptional activity by transcriptional activation analysis. Compared to wild-type plants, over-expression of *DoDELLA1* in tobacco resulted in dwarf and late-flowering or no flowering plants. The contents of GA_1 and GA_3 exhibited a decreasing trend in transgenic tobacco, and the expression levels of GA metabolism-related genes displayed a decreasing trend. Moreover, GA_3 treatment rescued the phenotype of *DoDELLA1* transgenic tobacco plants, and *DoDELLA1* transgenic tobacco plants had feedback and feed-forward mechanism of GA control, whereby bioactive GA reduced GA synthesis genes expression level and increased GA deactivation genes expression level. Our results suggest that *DoDELLA1* is involved in the regulation of transgenic tobacco plant growth and development by regulating GA_3 level via the regulation of the expression of GA metabolism-related genes.

Keywords Gibberellins · DELLA protein · *DoDELLA1* · GA biosynthesis and signaling · Tobacco phenotype

Introduction

Gibberellins (GAs) are plant hormones that regulate the growth and development of plants, including seed germination, stem elongation, leaf expansion, flower induction and development, and fruit set. GA is a large group of tetracyclic diterpenoid carboxylic acid synthesized from 2-oxoglutarate-dependent dioxygenases. The GA20-oxidases (GA20ox) and GA3-oxidases (GA3ox) mediate the last stage of GA biosynthesis by precursor GA12, which controls the levels of bioactive GAs (GA_1, GA_3, GA_4, etc.). Bioactive GAs levels can be deactivated by GA2-oxidases (GA2ox) (Hedden and Thomas 2012; Salazar-Cerezo et al. 2018). *JcGA2ox* transgenic plants led to lower GAs levels and displayed a typical dwarf phenotype in Arabidopsis(Hu et al. 2017). In pea, the *PsGA3ox1* overexpression plants had higher GAs concentrations, while altering GA biosynthesis and catabolism gene expression and plant phenotype(Reinecke et al. 2013). The GA deficient ga1-3 mutant and *AtGA2ox1* overexpression plants had a high

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expression level of DELLA protein(Middleton et al. 2012). The expression of GA20ox, GA3ox and GA2ox are maintained by a feedback regulation to respond to levels of bioactive GAs, which through development and environmental stimuli(Fukazawa et al. 2017).

The DELLA protein subfamily belongs to a large GRAS (named after GIBBERELLIN INSENSITIVE [GAI], REPRESSOR OF *gal*-3 [RGA], and SCARECROW [SCR]) transcription factor family with repressor activity(Daviere and Achard 2013; Hauvermale et al. 2012). Arabidopsis has five DELLA proteins: GAI (GAIN SENSITIVE), RGA (REPRESSOR-OF-GA), RGL1 (RGA-LIKE1), RGL2 (RGA-LIKE2), and RGL3 (RGA-LIKE3). Previous studies demonstrated that GAI, RGL1 and RGA play an essential role in regulating hypocotyl growth, stem and root elongation(Zheng et al. 2018; Dill and Sun 2001). *PmRGL2* in poplar was a negative regulator of GA responses that regulated bud dormancy and plant height(Lv et al. 2018). In oilseed rape, DELLA protein also negatively regulated plant height through GA(Zhao et al. 2017; Liu et al. 2010). In tomato, *SlGAI* could mediate the control of leaf form(Jasinski et al. 2008), *SlDELLA* operated as a growth repressor during fruit development by removing the response to GA and repressing cell expansion(Marti et al. 2007). Therefore, DELLA protein has the potential to actively coordinate plant growth and development.

DELLA protein is a key transcription factor in the GA signaling pathway (Daviere et al. 2008), which acts as a repressor of plant growth(Dill et al. 2001; Silverstone et al. 1997). The GA-GID1 (Gibberellin Insensitive Dwarf)-DELLA complexes are polyubiquitinated and degraded by 26 S proteasome(Dill et al. 2004; Ueguchi-Tanaka et al. 2007b; Murase et al. 2008; Ariizumi et al. 2011; Achard and Genschik 2009). It has been shown that DELLAs can regulate the expression of GA biosynthesis and catabolism genes. *AtGA20ox2* and *AtGA3ox1* were identified as targets of DELLA, which activate the expression of these genes(Zentella,2007). *SlDELLA* activated the expression of GA biosynthesis genes GA20ox1 and GA3ox1 in tomato(Hu et al. 2018). The DELLA-GAF1 (GAI-ASSOCIATED FACTOR1) complex was the main component in GA feedback regulation of GA20ox2 and GA3ox1(Wen et al. 2021).

Up to date, it is little known about the DELLA gene involving tuber morphogenesis in tuber crops. In potato, GA modulated host defense in response to phytoplasma infection via DELLA and SA signaling pathways(Ding et al. 2013). In cassava, *MeDELLA* silenced plants decreased the resistance to cassava bacterial blight, and *MeDELLA* and *MeGAI* were involved in GA signal resistance to drought stress (Li et al. 2018b). The involvement of GA_3 in yam tuber enlargement and yield was described(Kim et al. 2003; Yoshida et al. 2008). In our previous reports, high-level endogenous GA_3 had been identified in the yam early tuber expansion stage, and the application of GA_3 promoted tuber expansion(Gong et al. 2015, 2016). This implies that GA_3 affects the development of yam tuber, and other components of the GA signaling cascade may regulate tuber development.

In our previous study, exogenous GA_3 increased tuber weight, causing a significant in Guihuai 16 and Guihuai 5 tuber yield(Gong et al. 2015), and we isolated the DELLA protein gene for yam tuber and functionally characterized it in phylogenetic analysis(Zhou et al. 2021). Expression patterns were shown the transcripts of *DoDELLA1* were in response to GA_3 treatment in leaf, stem and tuber. In this study, a transgenic method was used to characterize DELLA protein function by overexpressing *DoDELLA1*. The possible mechanism underlying the regulation of *DoDELLA1* in plant growth and development was studied.

Materials and methods

Field experiment and sampling

The yam cultivars Guihuai 16 (GH16, with long and small tuber) and Guihuai 5 (GH5, with large tuber) were planted at the farm of Guangxi University (Fig.S1). Its healthy tubers' germination and planting patterns were as previously described(Gong et al. 2015). Young leaves and stems were defined as those borne on the first, second, third and fourth nodes (from the shoot tip), while mature leaves and stems were sampled from the 15th node after field planting for approximately 40d. Tuber samples were collected at tuber initiation, expansion and mature stage after field planting for approximately 40d, 90d, and 150d, respectively. Exogenous GA_3 and GA biosynthesis inhibitor paclobutrazol (PP_{333}) at 200 mg/L treatments were done as previously described(Gong et al. 2016), and summarized as below. The treatments were sprayed on the leaves of GH16 on June 20, and GH5 on July 20. Water-treated served as the control. All treatments were replicated three times, using 60 plants as a plot and arranged in a randomized complete block design. Mature leaves, mature stems and tubers were sampled 30d after treatment. Twelve plants were selected randomly from every repetition each time. All samples were immediately frozen in liquid nitrogen, and stored at -80°C. All samples were collected before 09:00 to minimize circadian effects on gene expression during 2019.

Yeast two-hybrid assay

The yeast transactivation assay was performed using Yeast and Transformants System(Hernandez-Garcia et al.

2019). The open reading frame and different fragments of *DoDELLA1* sequences were amplified and fused into the NdeI-BamHI sites of the pGBKT7 bait vector (GAL4 binding-domain, DBD). Primer pairs used were listed in Table S1. pGBKT7-53 and pGADT7-T vector acted as a positive control, pGBKT7-Lam and pGADT7-T vector acted as a negative control. Vectors (100ng) were introduced into Y2H gold yeast strains and cultured on SD medium without Leu containing AbA and X-α-gal at 30°C for 3d.

Plant materials, plasmid constructions, plant transformation and growth conditions

Tobacco plants (*Nicotiana tabacum*) were grown in tissue culture under a day photoperiod (14 h light and 8 h darkness) on half-strength Murashige and Skoog's (MS) medium containing 3%(w/v) sucrose. pBI121-*DoDELLA1*, which is a fragment cloned version of the yam DELLA1 gene, together with a cauliflower mosaic virus(CaMV) 35 S promoter and a nopaline synthase gene (NOS) terminator. Primer pairs were listed in Table S1. Tobacco leaf discs were infected with *Agrobacterium tumefaciens* strain AH105 harboring pBI121-*DoDELLA1*. The transformed plants were selected on MS medium containing 300 mg L^{-1} kanamycin and 500 mg L^{-1} claforan, and transferred to fresh medium monthly. Transgenic plants were transferred to pots filled containing potting soil with nutrients and moved to an environmental chamber (24°C, 16 h light/8 h dark). Independent transgenic seeds were germinated on MS medium containing 100 mg L^{-1} kanamycin and were cultured at the chamber(24°C, 16 h light/8 h dark). After a two-week selection, kanamycin-resistant plantlets were transferred to a fresh medium, followed by one month of culture under the same conditions. The plants were transferred to the soil under the same conditions for the isolation of homozygous line. T1 plants were self-pollinated to obtain the T2 generation. T2 seedlings were tested by kanamycin segregation, and a 3:1 segregation ratio was obtained for T3 generation. Homozygous lines were detected by kanamycin segregation and were determined by qRT-PCR (primer listed in Table S2). A total of six independent transgenic T3 lines were obtained (Fig.S2).

Quantitative real-time PCR analyses

Total RNA was extracted using the MiniBEST reagent (TaKaRa, Dalian, China), and reverse transcribed into cDNA using the PrimeScript RT reagent Kit (TaKaRa, Dalian, China). The expression levels of *DoDELLA1*, *NtGA20ox1-1*, *NtGA20ox1-3*, *NtGA3ox4-1*, *NtGA2ox1-3*, *NtGA2ox1-8* and *NtGA2ox2-3* were measured by quantitative real-time PCR using SYBR Green Supermix on the CFX96 Real-Time PCR Detection System (BIO-RAD), according to the manufacturer's protocol (primer listed in Table S2). All reactions were performed in triplicate. The $2^{-\Delta\Delta Ct}$ method was used to estimate relative expression level, and *DoActin* was used as internal controls for the yam tuber sample, *NtActin* was used as internal controls for different tobacco plants. Young leaves and stems were defined as those borne on the first, second, third and fourth nodes (from the shoot tip), while mature leaves and stems were sampled from the 15th node after field planting for approximately 20d as a control sample, respectively. The tuber control sample was collected after field planting for approximately 30d. The tobacco control sample was collected after germination.

Transgenic tobacco phenotype analyses

The wild-type and the third generations of transgenic tobacco plants were grown on agar medium, transplanted to the soil after germination. Samples were collected at 30d, 60d, and 120d after transplanting to the soil. Exogenous GA_3 and PP_{333} at 200 mg/L were sprayed at 30d, respectively. Each tobacco plant was used to measure plant height, internode length, leaf length and width, flower and fruit. Phenotypic measurements of tobacco plants were undertaken using three independent lines (three plants from each line). All phenotype analyses were collected from 30 plants, using Student's t-test. Mature, fully expanded functional leaves of wild-type and transgenic tobacco were collected during the growth period for the measurement of hormone levels. All samples were collected before 09:00 to minimize circadian effects on gene expression and hormone contents. The extraction method and quantitation of endogenous hormone were carried out as previously described(Gong et al. 2016). The extracted and purified samples were subjected to high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis.

Results

Expression patterns of DoDELLA1 in yam

In a previous study, GH5 and GH16 are known for reproducible individual differences in the response of stem and tuber to GA_3 (Fig.S1), and we cloned the full-length cDNA sequence of *DoDELLA1* (MV377793). *DoDELLA1* was redundantly expressed in all tissues of GH5 and GH16. *DoDELLA1* had a higher expression level in GH5 than in GH16 except in mature stems and initiation stage tuber without significant difference. *DoDELLA1* had a higher expression level in mature leaves, young stems and expansion stage tuber, but had lower expression in mature stems

and mature stage tuber in GH5. In GH16, the *DoDELLA1* expression level had the highest in young stems compared to other tissues, and the lowest expression in mature stage tuber. Interestingly, expression levels of *DoDELLA1* increased in the expansion stage and gradually declined with tuber development in GH5, but decreased gradually in GH16 with tuber development (Fig. 1 A).

GA_3 treatment down regulated *DoDELLA1* expression levels in leaf, stem and tuber of two cultivars, while PP_{333} treatment enhanced *DoDELLA1* expression levels except for stem. However, the degree of change in response to GA_3 and PP_{333} of leaf and tuber was higher than that of stem in two cultivars (Fig. 1B).

Transcriptional activation of DoDELLA1 N-terminal domain

We examined the transcriptional activation of DoDELLA1 using yeast expression system. Yeast strain Y2H Gold was transformed with a plasmid carrying the DNA-binding domain of the yeast GAL4 transcription factor (DBD_{GAL4}) fused with different fragments of DoDELLA1 protein (Fig. 1 C). Yeast cells carrying full-length DBD_{GAL4}-DoDELLA1 (plasmid D) grew blue in the SDO/X/A medium. To further analyze which conserved domain of DoDELLA1 is responsible for the transcriptional activation, we segmented the DoDELLA1 further into 231-634aa (plasmid d1), 285-634aa (plasmid d2), 114-634aa (plasmid d3), 35-284aa (plasmid d4) and 35-113aa (plasmid d5). Yeast cells carrying plasmid d3, plasmid d4 and plasmid d5 grew blue in SDO/X/A medium (Fig. 1D), suggesting that DELLA and VHYNP domain are involved in transcriptional activity, LH, VHIID and SAW domain are not involved in transcriptional activity.

The changes of characteristics of phenotype, physiology and gene expression in *DoDELLA1* transgenic tobacco line

DoDELLA1 transgenic tobacco lines were used to evaluate the role of *DoDELLA1*. The morphological characteristics of independent transgenic lines were obtained and *DoDELLA1* expression levels were detected in each transgenic tobacco line (Fig. 2 and Fig.S3). Compared to wild-type tobacco plants, over-expression *DoDELLA1* transgenic tobacco plants showed obvious dwarfing and late flowering. *DoDELLA*1-2, *DoDELLA1*-3 and *DoDELLA1*-4 lines showed more serious dwarf and no flowering. The expression

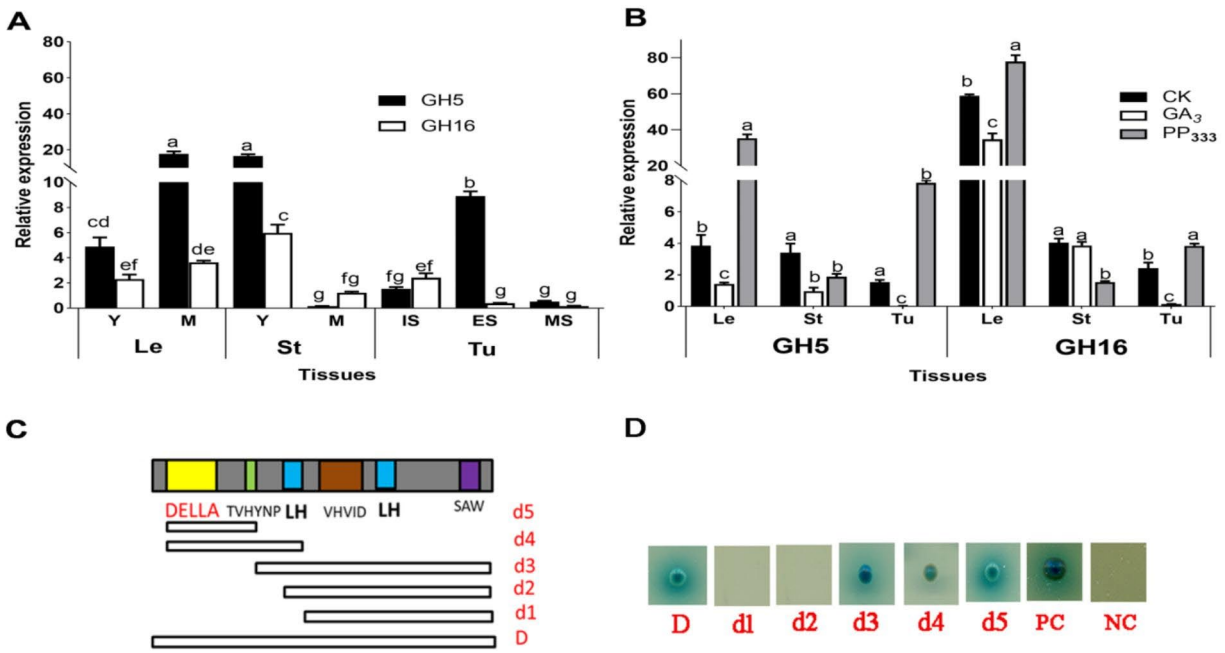

Fig. 1 Expression levels and transcriptional activation analysis of *DoDELLA1* in two yam cultivars. (A) Expression levels of *DoDELLA1*. (B) Effects of GA_3 and PP_{333} treatments on expression level of *DoDELLA1*. Transcripts accumulation was determined using qRT-PCR on three biological and three technical replicates. Letters indicate significantly different (Student's t-test; $P < / > 0.05$). Le, leaf; St, stem; Tu, tuber; Y, young; M, mature; IS, initiation stage; ES, expansion stage; MS, mature stage. (C) Schematic representation of the constructs used for transcriptional activation assay in yeast. (D) Transcriptional activation assay in yeast. PC: positive control, NC: negative control

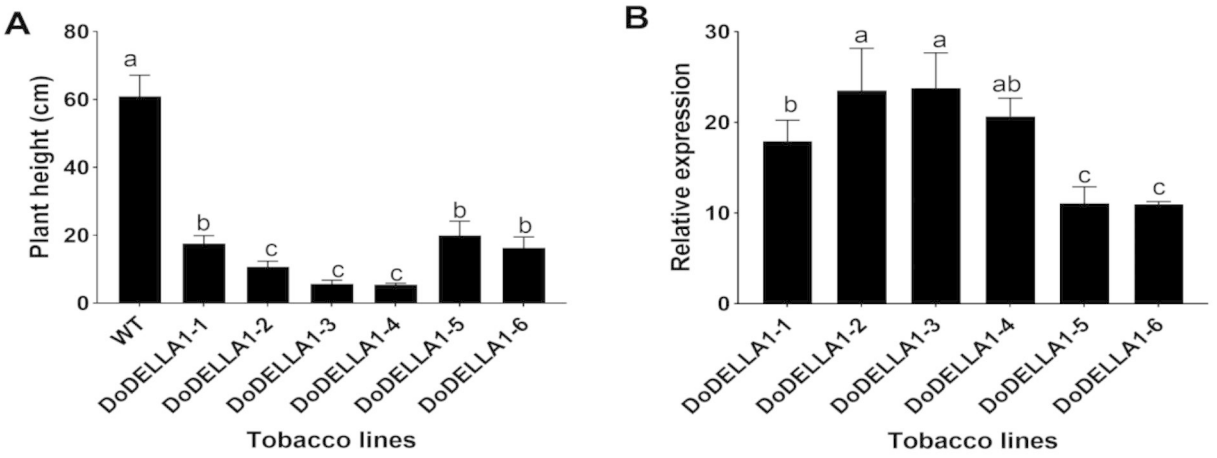

Fig. 2 Plant height (A) and *DoDELLA1* expression (B) in transgenic tobacco lines and wild-type (WT) tobacco. Plant height measurements are the means (± SE) of 30 seedlings. Transcripts accumulation was determined using qRT-PCR on three biological and three technical replicates. Letters indicate significantly different (Student's t-test; P < / > 0.05)

levels of *DoDELLA1* in *DoDELLA1*-2, *DoDELLA1*-3 and *DoDELLA1*-4 lines were higher than other lines, suggesting that the dwarfing and no flowering phenotype of transgenic plants was related to the expression level of *DoDELLA1*. Since *DoDELLA1*-2, *DoDELLA1*-3 and *DoDELLA1*-4 did not blossom and could not harvest seeds, we selected the *DoDELLA1*-1 line in T3 plants for further research in this experiment.

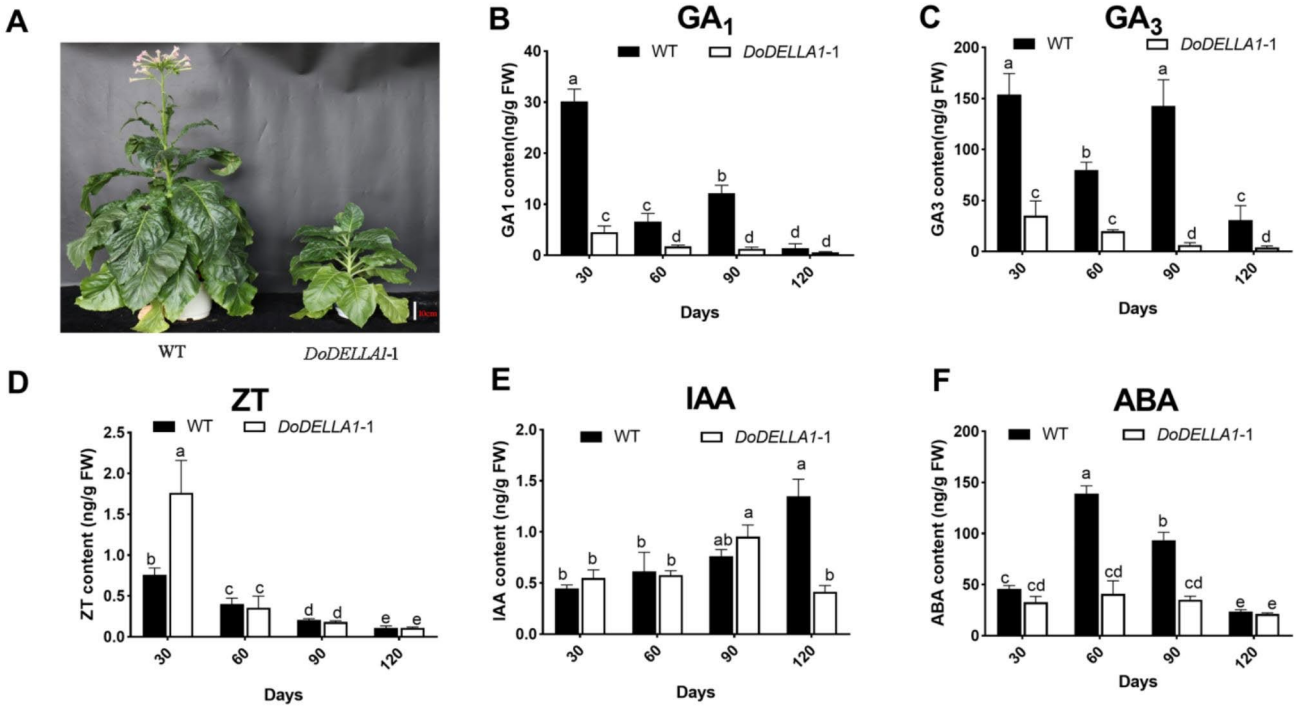

Fig. 3 Phenotypes and hormone contents in wild-type (WT) and *DoDELLA1*-1 tobacco line. A. Phenotypes of two tobacco lines at 120-day-old, bar = 10 cm. B-F. Hormone contents (GA_1, GA_3, ZT, IAA, ABA) of two tobacco lines in different developmental stages. Hormone accumulation was determined using HPLC-MS/MS on three biological and three technical replicates. Letters indicate significantly different (Student's t-test; P < / > 0.05)

The *DoDELLA1*-1 transgenic plants exhibited significant dwarfism, compact leaf type, and late flowering after 120d of growth (Fig. 3 A). The height of *DoDELLA1*-1 transgenic plants were 44.22% shorter than that in wild-type plants (Table 1). Besides, the number of internode and leaf in *DoDELLA1*-1 transgenic plants was 30.08% and 43.66% lower than wild-type plants, respectively. The weight of fruit was 33.33% lighter than that of wild-type plants. It shows that over-expression of *DoDELLA1*-1 in tobacco inhibits plant growth.

In the wild-type tobacco plants, the content of endogenous GA_1 and GA_3 showed two obvious peaks at 30d and 90d, respectively. GA_1 and GA_3 contents were the highest at 30d, which were 30.18 ng/g and 153.79 ng/g, respectively (Fig. 3B, C). ZT (Zeatin) content was the highest at 30d, which was 0.75ng/g, and gradually decreased with growth

Table 1 Vegetative and reproductive growth characteristics of wild-type (WT) and *DoDELLA1*-1 tobacco lines under GA_3 and PP_{333} treatment

	WT Control	WT +GA_3	WT +PP_{333}	*DoDELLA1-1* Control	*DoDELLA1-1* +GA_3	*DoDELLA1-1* +PP_{333}
Plant height/cm	70.67 ± 5.46b	82.8 ± 4.14a	31.13 ± 3.14d	49 ± 2.52c	70.8 ± 5.81b	24.75 ± 3.20d
Internode length/cm	1.43 ± 0.17c	4.90 ± 0.15a	0.79 ± 0.07de	1.21 ± 0.09 cd	2.30 ± 0.23b	0.55 ± 0.10e
Internode number	40 ± 0.82a	26 ± 1.48c	26.25 ± 1.93c	30.75 ± 0.93b	34.4 ± 1.11b	24.25 ± 2.17c
Stem diameter/cm	4.08 ± 0.15a	2.06 ± 0.04c	3.40 ± 0.36b	3.92 ± 0.13a	3.40 ± 0.10b	4.00 ± 0.35a
Leaf length/cm	31 ± 1.53a	21.82 ± 1.16b	21.95 ± 0.57b	22.625 ± 1.16b	31.26 ± 0.68a	14.55 ± 1.17c
Leaf width/cm	14.35 ± 0.70a	5.02 ± 0.29d	10.93 ± 0.44b	11.60 ± 0.20b	14.20 ± 0.46a	8.90 ± 0.47c
Leaf number	51.00 ± 3.81a	30.00 ± 1.41c	28.00 ± 0.91c	35.50 ± 1.04b	36.40 ± 0.68b	27.25 ± 2.06c
Fruit weight/g	0.36 ± 0.02a	0.37 ± 0.03a	0.07 ± 0.01c	0.27 ± 0.02b	0.40 ± 0.02a	0.05 ± 0.01c
Fruit number	79.50 ± 4.50b	76.33 ± 5.78b	27.25 ± 3.68c	75.5 ± 6.07b	117.00 ± 4.63a	10.00 ± 2.94d
Flower number	115.00 ± 5.00b	106.60 ± 14.52b	44.75 ± 5.56c	109.25 ± 13.62b	189.40 ± 16.73a	25.00 ± 5.40c
Flower time/day	185.75 ± 9.39c	234.5 ± 1.55ab	198 ± 4.93c	221.9 ± 4.85b	197 ± 5.51c	253 ± 11.78a

Plants were grown in LD photoperiod divided into three groups: control (water), sprayed with 200 mg/L of GA_3 (+GA_3) and PP_{333} (+PP_{333}), respectively. The measurements were taken from adult plants that had ~ 10% shattered seeds. Flowering time was scored upon the emergence of first flower. The bars represent the mean ± SE. of approximately 30 plants. Statistical differences were assessed using Student's t-test. Letters indicate significantly different (Student's t-test; P < / > 0.05)

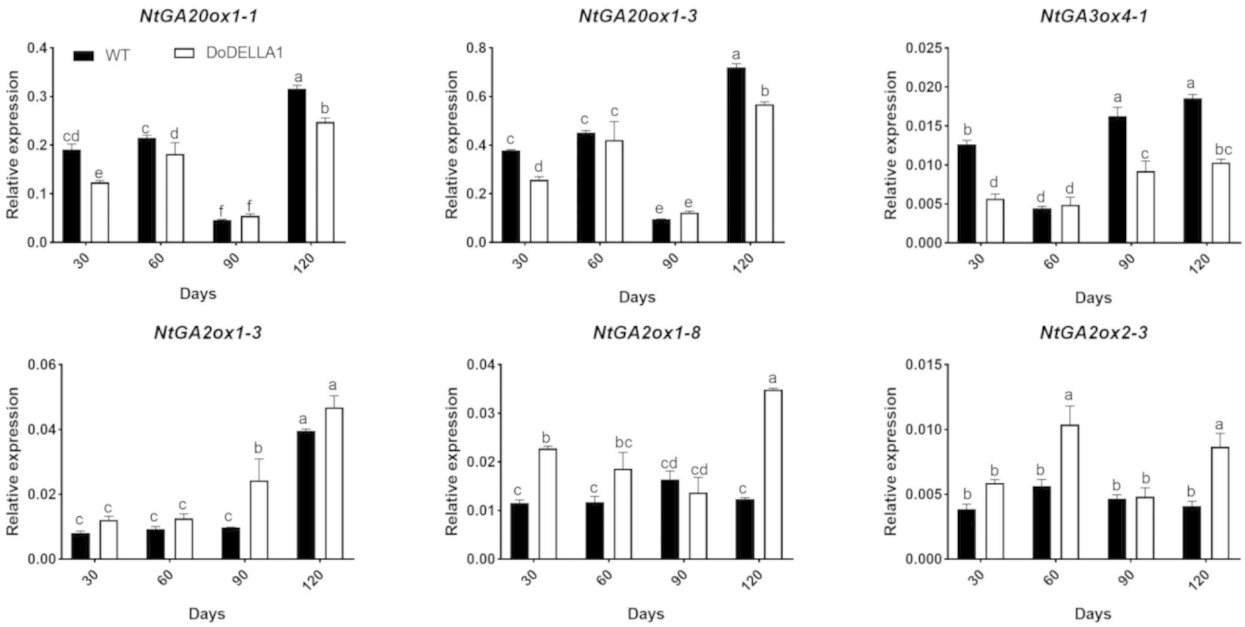

Fig. 4 The expression levels of tobacco GA-metabolism and signaling-related genes in wild-type (WT) and *DoDELLA1*-1 tobacco lines in different developmental stages. Transcripts accumulation was determined using qRT-PCR on three biological and three technical replicates. Letters indicate significantly different (Student's t-test; P < / > 0.05)

(Fig. 3D). IAA (Auxin) content gradually increased with growth, reaching the highest level of 1.34 ng/g at 120d (Fig. 3E). ABA (Abscisic acid) content increased first and then decreased with the growth, reaching the highest at 60d (Fig. 3 F).

In the *DoDELLA1*-1 transgenic tobacco plants, GA_1, GA_3 and ZT contents were the highest at 30d, which were 4.56 ng/g, 35.27 ng/g and 1.76 ng/g, respectively, and gradually decreased with growth (Fig. 3B, C). IAA content increased first and then decreased with the growth, reaching the highest at 90d, which was 0.95 ng/g (Fig. 3E). ABA content changed little at 30d, 60d and 90d, but decreased at 120d (Fig. 3 F). Compared to wild-type tobacco plants, GA_1 and GA_3 contents were significantly lower in the *DoDELLA1*-1 transgenic tobacco plants over 30-120d (Fig. 3B, C), IAA contents were significantly lower at 120d (Fig. 3E), ABA contents were significantly lower at 60d and 90d, and ZT contents were significantly higher at 30d (Fig. 3 F).

The expression of some tobacco genes involved in GA metabolism were assessed (Fig. 4). The expressions of *NtGA20ox1-1*, *NtGA20ox1-3* and *NtGA3ox4-1* were exhibited similar expression patterns in wild-type and *DoDELLA1*-1 transgenic tobacco plants, it decreased first and then increased along with the growth. *NtGA2ox1-3* expression increased in both tobacco lines. *NtGA2ox1-8* and *NtGA2ox2-3* expression tended to be stable in wild-type lines. *NtGA2ox1-8* expression decreased first and then increased in *DoDELLA1*-1 transgenic lines, while *NtGA2ox2-3* expression followed a down-up-down pattern. The expressions of *NtGA20ox1-1*, *NtGA20ox1-3* and *NtGA3ox4-1* were lower in *DoDELLA1*-1 transgenic lines than in wild-type lines. While *NtGA2ox1-3*, *NtGA2ox1-8* and *NtGA2ox2-3* enhanced the expression

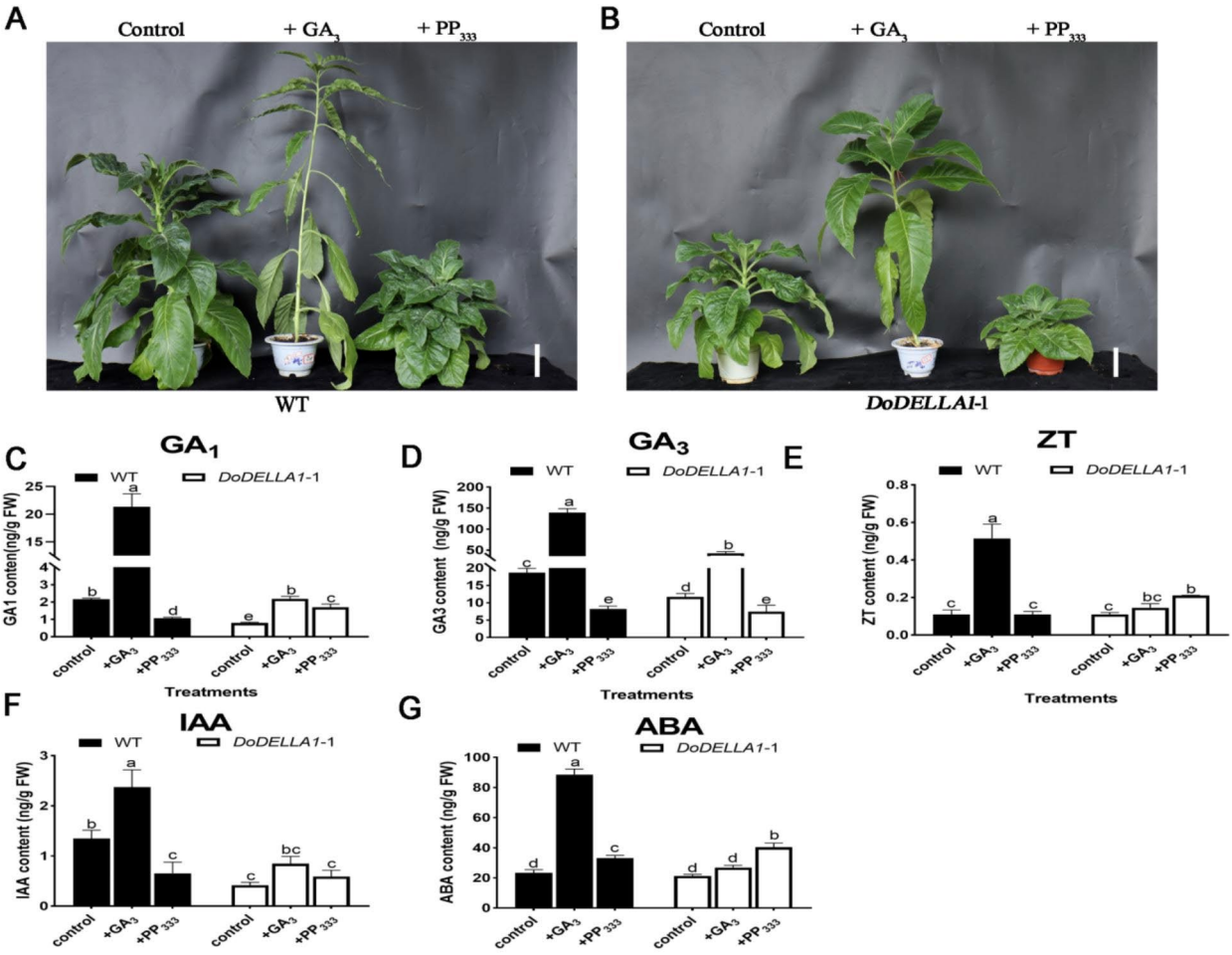

Fig. 5 Phenotypes and hormone contents of wild-type (WT) and *DoDELLA1*-1tobacco lines under GA_3 and PP_{333} treatments. A-B. Phenotypes of two tobacco lines at 120-day-old, bar = 10 cm. C-G. Hormone contents (GA_1, GA_3, ZT, IAA, ABA) of two tobacco lines at 120-day-old. Hormone accumulation was determined using HPLC-MS/MS on three biological and three technical replicates. Letters indicate significantly different (Student's t-test; P < / > 0.05)

levels in *DoDELLA1*-1 transgenic lines compared to wild-type lines. These results suggest that the over-expression of *DoDELLA1-1* decreased the levels of GA_1 and GA_3 in transgenic tobacco plants by down regulating the expressions of *NtGA20ox1-1*, *NtGA20ox1-3*, *NtGA3ox4-1*, and up regulating the expressions of *NtGA2ox1-3*, *NtGA2ox1-8* and *NtGA2ox2-3*. It shows that *DoDELLA1-1* can regulate the expressions of GA synthesis-related genes.

Effect of exogenous $GA_3$and PP_{333}on the characteristics of phenotype, physiology and gene expression in*DoDELLA1*-1 transgenic tobacco lines

The *DoDELLA1*-1 transgenic and wild-type tobacco plant were sprayed 200 mg/L exogenous GA_3 and PP_{333} at 30d after transplanting to the greenhouse (Table 1; Fig. 5 A). Compared to wild-type plants without treatment, wild-type plants exhibited slender growth associated with slender internode, stem and leaf, while plant height was enhanced by 17.16%, and delayed flowering by GA_3 treatment. Wild-type plants exhibited compact growth concomitant with a severe decrease in the height, stem diameter, internode length and number, leaf length, width and number, fruit weight and number, flower number by PP_{333} treatment. GA_3 treatment rescued to a certain extent *DoDELLA1*-1 transgenic plant height mainly due to extending internode length with no considerable changes in internode number. Moreover, GA_3 application restored leaf type, flower number, flowering time, fruit number and weight. Compared to the untreated *DoDELLA1*-1 transgenic lines, GA_3 application caused a significant increase in internodes length, leaf length and width, flower number, fruit weight and number, while plant height was enhanced by 44.48%, and precocious flowering in *DoDELLA1*-1 transgenic lines. PP_{333} treatment showed the strongest compact growth phenotype in *DoDELLA1*-1 transgenic lines.

Compared to untreated wild-type, GA_3 treatment significantly increased GA_1, GA_3, ZT, IAA and ABA contents by 8.85 fold, 6.46 fold, 3.71 fold, 0.76 fold and 2.78 fold in the wild-type plants, respectively (Fig. 5). PP_{333} treatment significantly decreased GA_1, GA_3 and IAA contents by 1.02 fold, 1.26 fold and 1.05 fold in the wild-type plants, but increased ABA content by 0.41 fold, respectively. Compared to untreated *DoDELLA1*-1 transgenic lines, GA_3 treatment significantly increased GA_1 and GA_3 contents by 1.76 fold and 2.63 fold in *DoDELLA1*-1 transgenic lines respectively, and increased ZT, IAA and ABA contents, but had no significant difference. PP_{333} treatment significantly decreased GA_3 content by 0.56 fold in *DoDELLA1*-1 transgenic lines, but increased GA_1, ZT and ABA contents by 0.51 fold, 0.92 fold and 0.90 fold, respectively. GA_1, ZT, IAA and ABA contents of *DoDELLA1*-1 transgenic lines after GA_3 treatment were restored to the wild-type plants level, and GA_3 content was significantly higher than the wild-type plants.

GA_3 treatment decreased *DoDELLA1* expression level, while PP_{333} treatment enhanced their expression level in *DoDELLA1-1* transgenic plants (Fig. 6 A). Furthermore, GA_3 and PP_{333} treatment decreased the expression levels of *NtGA20ox1-1*, *NtGA20ox1-3*, *NtGA2ox1-3* and *NtGA2ox1-8* in wild-type plants respectively, and GA_3 was more effective than PP_{333}. GA_3 treatment decreased significantly the expression levels of *NtGA20ox1-1*, *NtGA20ox1-3*, *NtGA2ox1-3* and *NtGA2ox1-8* in *DoDELLA1*-1 transgenic plants, increased significantly the expression levels of *NtGA3ox4-1* and *NtGA2ox2-3*, respectively. PP_{333} treatment increased significantly the expression levels of *NtGA20ox1-1*, *NtGA20ox1-3* and *NtGA2ox2-3* in *DoDELLA1*-1 transgenic plants, decreased significant *NtGA2ox1-3* and *NtGA2ox1-8* expression, respectively (Fig. 6B).

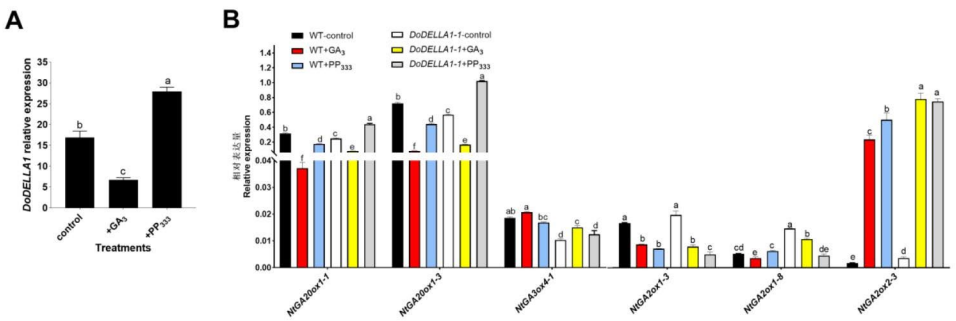

Fig. 6 The expression levels of genes in two tobacco lines under GA_3 and PP_{333} treatments. (A) The expression levels of *DoDELLA1* in *DoDELLA1-1* tobacco lines. (B) The expression levels of tobacco GA metabolism-related genes in wild-type (WT) and *DoDELLA1-1* tobacco lines. Transcripts accumulation was determined using qRT-PCR on three biological and three technical replicates. Letters indicate significantly different (Student's t-test; $P < / > 0.05$)

Discussion

DELLA proteins, which belong to the transcription factor in GA-dependent growth processes, have been reported in plant species, and designated as *SLR*1 in rice (*Oryza sativa*), *DWARF8* (*D*8) and *DWARF9* (*D*9) in maize (*Zea mays*), *MhGAI* in tea (*Malus hupehensis*), *Rht* in wheat (*Triticum aestivum*), and *FvRGA1* in strawberry (*Fragaria vesca*)(Li et al. 2018a; Hirano et al. 2012; Jusovic et al. 2018; Wang et al. 2012; Lawit et al. 2010). Previous studies have indicated that DELLA proteins play an important role in plant growth, such as plant height, stem elongation, seed germination, floral and root development(Vera-Sirera et al. 2016). In this study, the differences in expression levels of *DoDELLA1* in two yam cultivars may be the main factor regulating the varietal differences. Although *DoDELLA1* studies have been conducted functional in GA signaling by DoGID1s-DoDELLA1 in yam(Zhou et al. 2021), the regulatory role in yam is poorly understood. Therefore, the functions of the DoDELLA1 protein were investigated in tobacco development.

A transcriptional activation function of the N-terminal domains of DoDELLA1

DELLA proteins are a subfamily of the plant-specific GRAS family of transcriptional regulators that mediates gibberellin signaling. DELLA proteins have a unique structure divided into two major domains. The C-terminal GRAS functional domain is characterized by several motifs: two leucine heptad repeats (LH), VHIID and SAW, the N-terminal GA perception domain has two specific motifs: the DELLA and TVHYNP domains(Daviere and Achard 2013; Hauvermale et al. 2012). In previous studies, DoDELLA1 has two highly conserved C-terminal and N-terminal domains, and GA_3-DoGID1s-DoDELLA1 interaction was established(Zhou et al. 2021), which suggests the DoDELLA1 may indeed establish functional in GA signaling. Interestingly, although DoDELLA1 was indeed establishing functional interactions with DoGID1s, the N-terminal domains of DoDELLA1 had transcriptional activation (Fig. 1D). Previously, it has been suggested that DELLA and TVHYNP domains that were essential for this activity and GA response(Daviere and Achard 2016; Sun et al. 2010; Hirano et al. 2012). Additionally, DELLA proteins have been reported to act as a transcriptional coactivator in certain development contexts. *AtGAI* binding *AtGAF* co-transcriptionally activated GA synthesis pathway *AtGA20ox*, *AtGA3ox* and *AtGID1b* expression to regulate GA homeostasis(Fukazawa et al. 2014, 2017).

***DoDELLA1*plays an important role in the growth regulation of tobacco**.

The DELLA proteins were considered as master negative regulators of GA signaling, due to the dwarfism phenotypes in the overexpression of function, and tall or slender phenotypes characterized the loss of function(Daviere and Achard 2016; Hauvermale et al. 2012). Different plant species have different numbers of DELLA proteins and regulate different plant phenotypes. *AtRGA* and *AtGAI* perturbed overall plants growth behavior, including rooting capacity, plants architecture, and general vegetative and reproductive growth (Dill and Sun 2001; Feng et al. 2008; de Lucas et al. 2008), *AtRGL2* was the major inhibitor of seed germination(Lee et al. 2002), *AtRGA*, *AtRGL1* and *AtRGL2* together exhibited a significant reduction in silique length, seed number and disorder flower structure(Cheng et al. 2004; Tyler et al. 2004). The DWARF8 (D8) and DWARF9 (D9) in maize showed the same severe phenotype(Lawit et al. 2010). Overexpressing *PslDELLA* in Arabidopsis had strong dwarf plant height, short internodes, smaller flower size and seeds(El-Sharkawy et al. 2017).

In this study, we determined 6 transgenic lines by qRT-PCR and the expression level of *DoDELLA1* was higher, while the plant height was a severe dwarf (Fig. 2). Wild-type plants displayed the typical phenotype, whereas *DoDELLA1* transgenic plants were shorter in stature and leaf, late-flowering or no flowering, suggesting that DoDELLA1 protein function is consistent with other plant DELLA protein, and perturb tobacco plants growth behavior. *SIDELLA* inhibited tomato fruit size, but exogenous GA_3 rescued its phenotype(Marti et al. 2007). GA treatment rescued to a certain extent *PslGAI* and *PslRGL* plants height mainly due to extending internode length(El-Sharkawy et al. 2017). In this study, GA_3 treatment was rescued to *DoDELLA1* transgenic plant height and leaf size (Table 1; Fig. 5). It suggests that *DoDELLA1* is phenotypically characterized for GA-dependent traits and in response to GA_3 treatment.

The GA_3 levels in *DoDELLA1*-1 transgenic lines are significantly lower than in wild-type lines (Fig. 5). The significant different accumulation of GA_3 levels can be due to higher transcription *DoDELLA1* still impact GA metabolism to decrease GA3 levels in *DoDELLA1*-1 transgenic lines (Fig. 6). PP_{333} treatment significantly increased GA_1, ZT and ABA contents (Fig. 5), but exhibited the strong compact growth phenotype in *DoDELLA1*-1 transgenic lines (Table 1; Fig. 5), which had opposite effects with GA_3 treatment. There are maybe two reasons. First, ABA content increased by PP_{333} treatment can inhibit the growth of *DoDELLA1*-1 transgenic lines. Second, DELLA proteins not only are degraded with the classical proteolytic-dependent pathway in GA signaling, but also other alternative pathways. Partly mutant alleles of *sly* could not germinate, as they impaired the degradation and accumulated high levels of DELLA, and overexpression of GID1

could rescue germination without DELLA degradation, in which the formation of the GA-GID1-DELLA complex could inactivate the DELLA protein without degrading it (Ariizumi et al. 2008). SPINDLY is an O-linked-N-acetylglucosamine transferase that regulated the activity of the DELLA protein through glycosylation in Arabidopsis, rice and barley(Filardo et al. 2009; Shimada et al. 2006; Swain et al. 2001).

Mechanism of*DoDELLA1*regulates tobacco growth.

Bioactive GA plays a crucial role in coordinating different plant growth, and DELLA proteins may regulate GA biosynthesis modulating the expression of corresponding genes(Zentella et al. 2007). In this study, it was found that GA_1 and GA_3 content decreased in over-expression *DoDELLA1*-1 transgenic tobacco over 30-120d, and the expressions of *NtGA20ox1-1*, *NtGA20ox1-3* and *NtGA3ox4-1* were lower, while those of *NtGA2ox1-3*, *NtGA2ox1-8* and *NtGA2ox2-3* were higher during the same time (Fig. 4). These findings are consistent with overexpression of *PmRGL2* in poplars was reduced *PmGA20ox2*, *PmGA3ox1* expression which reduced the biosynthesis of GA_4 to release bud dormancy(Lv et al. 2018). *AtGA20ox2* and *AtGA3ox1* were identified as targets of DELLA, which activate the expression of these genes(Zentella,2007). *SlDELLA* might activate the expression of GA biosynthesis genes GA20ox1 and GA3ox1 in tomato fruit initiation(Hu et al. 2018). The DELLA-GAF1 complex was the main component in GA feedback regulation of GA20ox2 and GA3ox1 to elaborate leaf margin formation in *Medicago truncatula* (Wen et al. 2021). It suggests that *DoDELLA1* is conserved and involved in the regulation of GA metabolism genes to impact GAs levels, which perturbs tobacco plants' growth.

The application of GA_3 increased endogenous GA_3 levels, and PP_{333} decreased endogenous GA_3 levels in wild-type and *DoDELLA1*-1 transgenic plants. In the meanwhile, GA_3 treatment decreased the expression levels of *NtGA20ox1-1*, *NtGA20ox1-3*, and PP_{333} treatment increased the expression levels of *NtGA20ox1-1*, *NtGA20ox1-3* in wild-type and *DoDELLA1* transgenic plants. It means that *DoDELLA1* transgenic plants have feedback and feed-forward mechanism, whereby bioactive GAs reduces GA synthesis and speeds up GA deactivation. *AtGAI* binding *AtGAF* co-transcriptionally activated GA synthesis pathway *AtGA20ox*, *AtGA3ox* and *AtGID1b* expression to regulate GA homeostasis through feedback(Fukazawa et al. 2014, 2017). The expression of *NtGA20ox1-1*, *NtGA20ox1-3* are maintained by a feedback regulation to respond to levels of bioactive GA_3 in DoDELLA1 transgenic plants. However, *NtGA3ox4-1*, *NtGA2ox1-3*, *NtGA2ox1-8* and *NtGA2ox2-3* expression levels were not consistent with feedback and feed-forward mechanism in wild-type and *DoDELLA1*-1 transgenic lines under GA_3 and PP_{333} treatment (Fig. 6). Auxin and brassinosteroid regulated GA3ox and GA2ox expression mediating GA content(Hedden and Thomas 2012). It is speculated that other hormones are involved in the regulation of GA metabolism process. The nature of the differential expression between wild-type and *DoDELLA1*-1 transgenic lines is yet unclear and will require further analyses.

DELLA protein alleles *Vvgai1* inhibited plant height, leaf and flower development, a DELLA-centered feedback mechanism that maintains GA homeostasis in *Vvgai1* transgenic soybean plants was found, but it was believed that the intricate interactions of DELLAs with numerous transcription factors control plant development and growth, which involved the growth of stem, leaf and flower, such as *VvGRF* (GROETH REGULATING FACTOR), *VvPIF1* (PHYTOCHROME INTERACTING FACTOR 1), *VvEXP1* (EXPANSIN), *VvCO* (CONSTANS) and *VvTFL1* (CENTRORADIALIS)(Arro et al. 2019). In this study, DoDELLA1 proteins reduced internode length and height in tobacco, and impacted GA metabolism genes to regulate GA_3 level. However, it could not be an identity that DoDELLA1 protein is involved in GA homeostasis regulation directly. Since previous studies indicate that DELLAs repress plant growth by binding to a wide variety of proteins or transcriptional regulators by GA signaling, while involved in other plant hormones. DELLA proteins interacted with ARF6 (AUXIN RESPONSE FACTOR), BZR1 (BRASSINAZOLE-RESISTANT1) and PIF4 (PHYTOCHROME INTERACTING FACTOR 4), which involved promoting hypocotyl cell elongation in *Arabidopsis thaliana* seedling growth by integrating GA, brassinosteroid, auxin, and light/temperature environmental stimuli(de Lucas et al. 2008; Feng et al. 2008; Gallego-Bartolome et al. 2012; Oh et al. 2014). *AtDELLA*, *AtABI3* (ABA INSENSITIVE 3), and *AtABI5* (ABA INSENSITIVE 5) formed a protein complex that bound the promoter and activated the transcription of target genes, such as SOMNUS (SOM), a C3H-type zinc finger that negatively regulated seed germination(Lim et al. 2013). In this study, GA_1, GA_3 and ABA contents were significantly reduced in the *DoDELLA1*-1transgenic lines, while GA_1, ZT, IAA and ABA contents were restored to the wild-type plants level after GA_3 treatment (Fig. 5). The mechanism of *DoDELLA1* regulating GA metabolism and signaling pathways in an integrated manner with other transcription factors by ZT, IAA and ABA, is still unknown, and needs further research.

Conclusions

In conclusion, our results suggest that *DoDELLA1*, a considered major negative regulator of GA signaling gene from

yam tuber, inhibited the growth and development of transgenic tobacco plants. The observed changes in the dwarf phenotype of the transgenic tobacco plants may result from GA content changes via the regulation of the expression of GA-related genes by *DoDELLA1*.

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Author contributions Y.Y.Z. performed the experiments and wrote the manuscript. Y.T.L., J.M.H and R.R.J performed some experiments. M.L., D.X. and J.Z. provided technical assistance and research guidance. A.Q.W. and L.F.H. designed the research and revised the manuscript.

Declarations The authors declare that they have no conflict of interest.

References

Achard P, Genschik P (2009) Releasing the brakes of plant growth: how GAs shutdown DELLA proteins. J Exp Bot 60(4):1085–1092. doi:https://doi.org/10.1093/jxb/ern301

Ariizumi T, Murase K, Sun TP, Steber CM (2008) Proteolysis-independent downregulation of DELLA repression in Arabidopsis by the gibberellin receptor GIBBERELLIN INSENSITIVE DWARF1. Plant Cell 20(9):2447–2459. doi:https://doi.org/10.1105/tpc.108.058487

Ariizumi T, Lawrence PK, Steber CM (2011) The role of two f-box proteins, SLEEPY1 and SNEEZY, in Arabidopsis gibberellin signaling. Plant Physiol 155(2):765–775. doi:https://doi.org/10.1104/pp.110.166272

Arro J, Yang Y, Song GQ, Zhong GY (2019) RNA-Seq reveals new DELLA targets and regulation in transgenic GA-insensitive grapevines. BMC Plant Biol 19(1):80. doi:https://doi.org/10.1186/s12870-019-1675-4

Cheng H, Qin L, Lee S, Fu X, Richards DE, Cao D, Luo D, Harberd NP, Peng J (2004) Gibberellin regulates Arabidopsis floral development via suppression of DELLA protein function. Development 131(5):1055–1064. doi:https://doi.org/10.1242/dev.00992

Daviere JM, de Lucas M, Prat S (2008) Transcriptional factor interaction: a central step in DELLA function. Curr Opin Genet Dev 18(4):295–303. doi:https://doi.org/10.1016/j.gde.2008.05.004

Daviere JM, Achard P (2013) Gibberellin signaling in plants. Development 140(6):1147–1151. doi:https://doi.org/10.1242/dev.087650

Daviere JM, Achard P (2016) A pivotal role of DELLAs in regulating multiple hormone signals. Mol Plant 9(1):10–20. doi:https://doi.org/10.1016/j.molp.2015.09.011

de Lucas M, Daviere JM, Rodriguez Falcon M, Pontin M, Iglesias Pedraz JM, Lorrain S, Fankhauser C, Blazquez MA, Titarenko E, Prat S (2008) A molecular framework for light and gibberellin control of cell elongation. Nature 451(7177):480–484. doi:https://doi.org/10.1038/nature06520

Dill A, Sun T (2001) Synergistic derepression of gibberellin signaling by removing RGA and GAI function in *Arabidopsis thaliana*. Genetics 159(2):777–785

Dill A, Jung HS, Sun TP (2001) The DELLA motif is essential for gibberellin-induced degradation of RGA. PNAS 98(24):14162–14167. doi:https://doi.org/10.1073/pnas.251534098

Dill A, Thomas SG, Hu J, Steber CM, Sun TP (2004) The Arabidopsis F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. Plant Cell 16(6):1392–1405. doi:https://doi.org/10.1105/tpc.020958

Ding Y, Wei W, Wu W, Davis RE, Jiang Y, Lee IM, Hammond RW, Shen L, Sheng JP, Zhao Y (2013) Role of gibberellic acid in tomato defence against potato purple top phytoplasma infection. Ann of Applied Biology 162(2):191–199. doi:https://doi.org/10.1111/aab.12011

El-Sharkawy I, Sherif S, Abdulla M, Jayasankar S (2017) Plum fruit development occurs via gibberellin-sensitive and-insensitive DELLA repressors. PLoS ONE 12(1):e0169440. doi:https://doi.org/10.1371/journal.pone.0169440

Feng SH, Martinez C, Gusmaroli G, Wang Y, Zhou JL, Wang F, Chen LY, Yu L, Iglesias-Pedraz JM, Kircher S, Schafer E, Fu XD, Fan LM, Deng XW (2008) Coordinated regulation of *Arabidopsis thaliana* development by light and gibberellins. Nature 451(7177):475–U479. doi:https://doi.org/10.1038/nature06448

Filardo F, Robertson M, Singh DP, Parish RW, Swain SM (2009) Functional analysis of *HvSPY*, a negative regulator of GA response, in barley aleurone cells and Arabidopsis. Planta 229(3):523–537. doi:https://doi.org/10.1007/s00425-008-0843-9

Fukazawa J, Teramura H, Murakoshi S, Nasuno K, Nishida N, Ito T, Yoshida M, Kamiya Y, Yamaguchi S, Takahashi Y (2014) DELLAs function as coactivators of GAI-ASSOCIATED FACTOR1 in regulation of gibberellin homeostasis and signaling in Arabidopsis. Plant Cell 26(7):2920–2938. doi:https://doi.org/10.1105/tpc.114.125690

Fukazawa J, Mori M, Watanabe S, Miyamoto C, Ito T, Takahashi Y (2017) DELLA-GAF1 complex is a main component in gibberellin feedback regulation of GA20 oxidase 2. Plant Physiol 175(3):1395–1406. doi:https://doi.org/10.1104/pp.17.00282

Gallego-Bartolome J, Minguet EG, Grau-Enguix F, Abbas M, Locascio A, Thomas SG, Alabadi D, Blazquez MA (2012) Molecular mechanism for the interaction between gibberellin and brassinosteroid signaling pathways in Arabidopsis. PNAS 109(33):13446–13451. doi:https://doi.org/10.1073/pnas.1119992109

Gong M, Luo H, Yuan H, Wei S, Yang X, He L (2015) Effects of exogenous gibberellin and paclobutrazol on tuber expansion and bulbil formation of Chinese Yam. Acta Horticulturae Sinica 42(6):1175–1184

Gong M, Luo H, Wang A, Zhou Y, Huang W, Zhu P, He L (2016) Phytohormone profiling during tuber development of Chinese yam by ultra-high performance liquid chromatography–triple quadrupole tandem mass spectrometry. J Plant Growth Regul 36(2):362–373. doi:https://doi.org/10.1007/s00344-016-9644-8

Hauvermale AL, Ariizumi T, Steber CM (2012) Gibberellin signaling: a theme and variations on DELLA repression. Plant Physiol 160(1):83–92. doi:https://doi.org/10.1104/pp.112.200956

Hedden P, Thomas SG (2012) Gibberellin biosynthesis and its regulation. Biochem J 444(1):11–25. doi:https://doi.org/10.1042/BJ20120245

Hernandez-Garcia J, Briones-Moreno A, Dumas R, Blazquez MA (2019) Origin of gibberellin-dependent transcriptional regulation by molecular exploitation of a transactivation domain in DELLA proteins. Mol Biol Evol 36(5):908–918. doi:https://doi.org/10.1093/molbev/msz009

Hirano K, Kouketu E, Katoh H, Aya K, Ueguchi-Tanaka M, Matsuoka M (2012) The suppressive function of the rice DELLA protein SLR1 is dependent on its transcriptional

activation activity. Plant J 71(3):443–453. doi:https://doi.org/10.1111/j.1365-313X.2012.05000.x

Hu YX, Tao YB, Xu ZF (2017) Overexpression of Jatropha gibberellin 2-oxidase 6 (*JcGA2ox6*) induces dwarfism and smaller leaves, flowers and fruits in Arabidopsis and Jatropha. Front Plant Sci 8:2103. doi:https://doi.org/10.3389/fpls.2017.02103

Hu J, Israeli A, Ori N, Sun TP (2018) The Interaction between DELLA and ARF/IAA mediates crosstalk between gibberellin and auxin signaling to control fruit initiation in tomato. Plant Cell 30(8):1710–1728. doi:https://doi.org/10.1105/tpc.18.00363

Jasinski S, Tattersall A, Piazza P, Hay A, Martinez-Garcia JF, Schmitz G, Theres K, McCormick S, Tsiantis M (2008) PROCERA encodes a DELLA protein that mediates control of dissected leaf form in tomato. Plant J 56(4):603–612. doi:https://doi.org/10.1111/j.1365-313X.2008.03628.x

Jusovic M, Velitchkova MY, Misheva SP, Borner A, Apostolova EL, Dobrikova AG (2018) Photosynthetic responses of a wheat mutant (Rht-B1c) with altered DELLA proteins to salt stress. J Plant Growth Regul 37(2):645–656. doi:https://doi.org/10.1007/s00344-017-9764-9

Kim SK, Lee SC, Shin DH, Jang SW, Nam JW, Park TS, Lee IJ (2003) Quantification of endogenous gibberellins in leaves and tubers of Chinese yam, *Dioscorea opposita* Thunb. cv. Tsukune during tuber enlargement. Plant Growth Regul 39(2):125–130. doi:Doi https://doi.org/10.1023/A:1022569506883

Lawit SJ, Wych HM, Xu DP, Kundu S, Tomes DT (2010) Maize DELLA proteins dwarf plant8 and dwarf plant9 as modulators of plant development. Plant and Cell Physiol 51(11):1854–1868. doi:https://doi.org/10.1093/pcp/pcq153

Lee SC, Cheng H, King KE, Wang WF, He YW, Hussain A, Lo J, Harberd NP, Peng JR (2002) Gibberellin regulates Arabidopsis seed germination via RGL2, a GAI/RGA-like gene whose expression is up-regulated following imbibition. Genes Dev 16(5):646–658. doi:DOI https://doi.org/10.1101/gad.969002

Li WJ, Zhang JX, Sun HY, Wang SM, Chen KQ, Liu YX, Li H, Ma Y, Zhang ZH (2018a) *FveRGA1*, encoding a DELLA protein, negatively regulates runner production in *Fragaria vesca*. Planta 247(4):941–951. doi:https://doi.org/10.1007/s00425-017-2839-9

Li X, Liu W, Li B, Liu G, Wei Y, He C, Shi H (2018b) Identification and functional analysis of cassava DELLA proteins in plant disease resistance against cassava bacterial blight. Plant Physiol Biochem 124:70–76. doi:https://doi.org/10.1016/j.plaphy.2017.12.022

Lim S, Park J, Lee N, Jeong J, Toh S, Watanabe A, Kim J, Kang H, Kim DH, Kawakami N, Choi G (2013) ABA-INSENSITIVE3, ABA-INSENSITIVE5, and DELLAs interact to activate the expression of SOMNUS and other high-temperature-inducible genes in imbibed seeds in Arabidopsis. Plant Cell 25(12):4863–4878. doi:https://doi.org/10.1105/tpc.113.118604

Liu C, Wang J, Huang T, Wang F, Yuan F, Cheng X, Zhang Y, Shi S, Wu J, Liu K (2010) A missense mutation in the VHYNP motif of a DELLA protein causes a semi-dwarf mutant phenotype in *Brassica napus*. Theor Appl Genet 121(2):249–258. doi:https://doi.org/10.1007/s00122-010-1306-9

Lv L, Huo X, Wen L, Gao Z, Khalil-Ur-Rehman M (2018) Isolation and role of *PmRGL2* in GA-mediated floral bud dormancy release in Japanese apricot (*Prunus mume Siebold* et Zucc.). Front Plant Sci 9:27. doi:https://doi.org/10.3389/fpls.2018.00027

Marti C, Orzaez D, Ellul P, Moreno V, Carbonell J, Granell A (2007) Silencing of DELLA induces facultative parthenocarpy in tomato fruits. Plant J 52(5):865–876. doi:https://doi.org/10.1111/j.1365-313X.2007.03282.x

Middleton AM, Ubeda-Tomas S, Griffiths J, Holman T, Hedden P, Thomas SG, Phillips AL, Holdsworth MJ, Bennett MJ, King JR, Owen MR (2012) Mathematical modeling elucidates the role of transcriptional feedback in gibberellin signaling. P Natl Acad Sci USA 109(19):7571–7576. doi:https://doi.org/10.1073/pnas.1113666109

Murase K, Hirano Y, Sun TP, Hakoshima T (2008) Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. Nature 456(7221):459–463. doi:https://doi.org/10.1038/nature07519

Oh E, Zhu JY, Bai MY, Arenhart RA, Sun Y, Wang ZY (2014) Cell elongation is regulated through a central circuit of interacting transcription factors in the Arabidopsis hypocotyl. eLife 3:e03031. doi:https://doi.org/10.7554/eLife.03031

Reinecke DM, Wickramarathna AD, Ozga JA, Kurepin LV, Jin AL, Good AG, Pharis RP (2013) Gibberellin 3-oxidase gene expression patterns influence gibberellin biosynthesis, growth, and development in pea. Plant Physiol 163(2):929–945. doi:https://doi.org/10.1104/pp.113.225987

Salazar-Cerezo S, Martinez-Montiel N, Garcia-Sanchez J, Perez YTR, Martinez-Contreras RD (2018) Gibberellin biosynthesis and metabolism: A convergent route for plants, fungi and bacteria. Microbiol Res 208:85–98. doi:https://doi.org/10.1016/j.micres.2018.01.010

Shimada A, Ueguchi-Tanaka M, Sakamoto T, Fujioka S, Takatsuto S, Yoshida S, Sazuka T, Ashikari M, Matsuoka M (2006) The rice SPINDLY gene functions as a negative regulator of gibberellin signaling by controlling the suppressive function of the DELLA protein, SLR1, and modulating brassinosteroid synthesis. Plant J 48(3):390–402. doi:https://doi.org/10.1111/j.1365-313X.2006.02875.x

Silverstone AL, Mak PY, Martinez EC, Sun TP (1997) The new RGA locus encodes a negative regulator of gibberellin response in *Arabidopsis thaliana*. Genetics 146(3):1087–1099

Sun X, Jones WT, Harvey D, Edwards PJB, Pascal SM, Kirk C, Considine T, Sheerin DJ, Rakonjac J, Oldfield CJ, Xue B, Dunker AK, Uversky VN (2010) N-terminal domains of DELLA proteins are intrinsically unstructured in the absence of interaction with GID1/gibberellic acid receptors. J Biol Chem 285(15):11557–11571. doi:https://doi.org/10.1074/jbc.M109.027011

Swain SM, Tseng TS, Olszewski NE (2001) Altered expression of SPINDLY affects gibberellin response and plant development. Plant Physiol 126(3):1174–1185. doi:https://doi.org/10.1104/pp.126.3.1174

Tyler L, Thomas SG, Hu J, Dill A, Alonso JM, Ecker JR, Sun TP (2004) Della proteins and gibberellin-regulated seed germination and floral development in Arabidopsis. Plant Physiol 135(2):1008–1019. doi:https://doi.org/10.1104/pp.104.039578

Vera-Sirera F, Gomez MD, Perez-Amador MA (2016) DELLA proteins, a group of GRAS transcription regulators that mediate gibberellin signaling. In: Plant Transcription Factors. pp 313–328. doi:https://doi.org/10.1016/b978-0-12-800854-6.00020-8

Wang SS, Liu ZZ, Sun C, Shi QH, Yao YX, You CX, Hao YJ (2012) Functional characterization of the apple *MhGAI1* gene through ectopic expression and grafting experiments in tomatoes. J Plant Physiol 169(3):303–310. doi:https://doi.org/10.1016/j.jplph.2011.09.012

Wen L, Kong Y, Wang H, Xu Y, Lu Z, Zhang J, Wang M, Wang X, Han L, Zhou C (2021) Interaction between the MtDELLA-MtGAF1 complex and MtARF3 mediates transcriptional control of MtGA3ox1 to elaborate leaf margin formation in *Medicago truncatula*. Plant Cell Physiol 62(2):321–333. doi:https://doi.org/10.1093/pcp/pcaa163

Yoshida Y, Ichikawa A, Takahashi M, Takahashi H, Kanda H, Kanahama K (2008) The effects of gibberellin concentration, treatment interval and commencement timing on the development of tubers and inflorescences in Chinese yam (*Dioscorea oppositifolia* L.) 'Ichoimo' plant. Paper presented at the Proceedings of the International Symposium on Endogenous and Exogenous Plant Bioregulators

Zentella R, Zhang Z-L, Park M, Thomas SG, Endo A, Murase K, Fleet CM, Jikumaru Y, Nambara E, Kamiya Y, Sun T-P (2007) Global analysis of DELLA direct targets in early gibberellin signaling in Arabidopsis. Plant Cell 19(10):3037–3057. doi:https://doi.org/10.1105/tpc.107.054999

Zhao B, Li H, Li J, Wang B, Dai C, Wang J, Liu K (2017) *Brassica napus* DS-3, encoding a DELLA protein, negatively regulates stem elongation through gibberellin signaling pathway. Theor Appl Genet 130(4):727–741. doi:https://doi.org/10.1007/s00122-016-2846-4

Zheng H, Zhang F, Wang S, Su Y, Ji X, Jiang P, Chen R, Hou S, Ding Y (2018) MLK1 and MLK2 coordinate RGA and CCA1 activity to regulate hypocotyl elongation in *Arabidopsis thaliana*. Plant Cell 30(1):67–82. doi:https://doi.org/10.1105/tpc.17.00830

Zhou Y, Li Y, Gong M, Qin F, Xiao D, Zhan J, Wang A, He L (2021) Regulatory mechanism of GA3 on tuber growth by DELLA-dependent pathway in yam (*Dioscorea opposita*). Plant Mol Biol 106:433–448. doi:https://doi.org/10.1007/s11103-021-01163-7