

Demonstration of improved plant growth and biomass by At1g promoter-controlled root-preferential expression of *AtGA20ox* gene

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Abstract

Background: Overexpression of *GA20 oxidase* gene has been a recent trend for improving plant growth and biomass. Constitutive expression of *GA20ox* has successfully improved plant growth and biomass in several plant species. However, the constitutive expression of this gene causes side-effects, such as reduced leaf size and stem diameters, etc. To avoid these effects, different tissue-specific promoters had been identified and employed for *GA20ox* overexpression. In this study, we demonstrate the potential of At1g, a root-preferential promoter, for *GA20ox* expression to enhance plant biomass.

Results: We examined the utility of At1g promoter to drive the expression of GUS (b-glucuronidase) reporter and *GA20ox* genes in tobacco and *Melia azedarach*. Histochemical GUS assays in tobacco showed that At1g was a root-preferential promoter whose expression was particularly strong in root tips. The ectopic expression of *AtGA20ox* gene under the control of At1g promoter showed the improved plant growth and biomass of both tobacco and *M. azedarach* transgenic plants compared to wild-type (WT) control plants. Stem length as well as stem and root fresh weights increased by up to 1.5-3 folds in transgenic tobacco and 2 folds in transgenic *M. azedarach*. Both tobacco and *M. azedarach* transgenic plants showed increases in the root xylem width, xylem over phloem ratio by 50%-100% as compared to WT plants. Importantly, no significant difference in the leaf shape and size was observed between At1g::*AtGA20ox* transgenic and WT plants. Moreover, transgenic *M. azedarach* showed a 135% increase in stem diameter even though no change was found in transgenic tobacco.

Conclusions: These results demonstrate the great utility of At1g promoter, when driving *AtGA20ox* gene, for growth and biomass improvements in woody plants and potentially some other plant species.

Background

Intense efforts have been made to improve plant growth and biomass production of important crops as well as forestry tree plants to meet the increased demand of global food, feed, fiber and energy [1–5]. Last two decades have witnessed plant growth and biomass improvement by transgenic expression of *GA20ox* gene in different plant species [6–15]. *GA20ox* is a key enzyme involved in gibberellin biosynthesis (GAs) pathway and directly controls bioactive GAs contents in plants [16–20]. Previous studies showed that overexpression of *GA20-oxidase* (*GA20ox*) conferred desirable phenotypes including accelerated stem growth, increased biomass production, enhanced fiber cell elongation, fostered early flowering, and decreased seed dormancy [6, 14, 21, 22].

Controlled *GA20ox* expression to achieve optimal bioactive GA contents in transgenic plants has gained a growing attention. The strong and constitutive 35S promoter has been widely used in plant transformation [23]. However, previous reports found that *GA20ox* overexpression under the control of constitutive promoters, particularly CaMV35S, caused undesirable phenotypes such as poor root growth, low numbers of adventitious roots, reduced stem diameter, small leaf area and delayed flowering due to the hyper-accumulation of bioactive gibberellins [7, 11, 14, 22, 24, 25]. The undesirable phenotypes were

eliminated when 35S promoter was replaced by a non-constitutive promoter such as CAD4 or DX15 [22, 26]. Thus, recent trend has been for identifying and employing suitable promoters to drive GA20ox expression in order to achieve desirable phenotypes.

At1g promoter, a 614 bp upstream sequence of the At1g73160 gene, was isolated from chromosome 1 of *Arabidopsis thaliana* previously [27]. This *Arabidopsis* genome locus encodes UDP-glycosyltransferase superfamily protein. The report showed that At1g73160 promoter was predicted to be root-specific expression based on sequence information and GUS assay. This promoter contains several different root motifs such as ASF1 MOTIF CAMV, ARFAT, OSE1, OSE2, RAV1AAT, SURE core and TAPOX1, which involved in root tissue expression. As At1g promoter was used to drive GUS gene, GUS staining was observed only in *Arabidopsis* roots with the primary root displaying stronger GUS expression than the secondary roots. However, the expression patterns of this promoter have not been fully characterized at molecular levels for the whole *Arabidopsis* plant. Therefore, further researches were needed to validate the function of At1g promoter for its utility in biotechnological applications and basic research.

In this research, we first ascertained the root-preferential expression of At1g promoter in transgenic tobacco using GUS (β -glucuronidase) reporter gene. Then, the ectopic AtGA20ox expression by At1g promoter showed desirable plant morphological alterations, including accelerated plant growth and increased biomass production in both transgenic tobacco and *M. azedarach*. Importantly, these desirable phenotypes were consistently observed in all At1g::AtGA20ox transgenic plants as compared to non-transgenic plants. This study demonstrates the great utility of the root-preferential At1g promoter to control AtGA20ox gene expression for plant growth and biomass improvement.

Results

Root-preferential expression of At1g promoter

Overexpression of AtGA20ox gene in tobacco under the controls of different promoters

The GUS gene under the control of CaMV35S or At1g promoter was cloned into the binary vector pBI121 which was used to transform tobacco cultivar K326 using *Agrobacterium* mediated method (Fig. S3). Transgenic tobacco plants of 35S::GUS and At1g::GUS constructs were randomly selected for GUS assays. The GUS gene expression indicated by histochemical blue staining was observed in all tested tissues including leaves, stems and roots of 35S::GUS transgenic lines (Fig. 1). In contrast, GUS expression was only found in roots, particularly in root tips, of At1g::GUS transgenic tobacco plants. In addition, expression of GUS gene was stronger in root tips of At1g::GUS than 35S::GUS transgenic tobacco. These results demonstrated the root-preferential expression of At1g promoter in tobacco, especially in root tips.

We further characterized At1g::AtGA20ox and 35S::AtGA20ox transgenic tobacco plants. Specific primers for AtGA20ox were used to confirm the presence of this transgene in transgenic lines. All tested plants showed clear PCR bands (1.2 kb) in the gel running results (Fig. 2a) indicating the transgene integration in tobacco genome. We used RT-PCR to determine transcript levels of AtGA20ox in different tissue including roots, stems and leaves of transgenic lines. A higher transcript level of AtGA20ox was found in 35S::AtGA20ox than that in At1g::AtGA20ox transgenic lines in all tested tissues. We also compared AtGA20ox expression levels in different tissues of transgenic tobacco. The 35S::AtGA20ox transgenic lines showed no significant difference in AtGA20ox expression among roots, leaves and stems. However, AtGA20ox expressions varied in root, stem and leaf tissue (in this descending order) of At1g::AtGA20ox transgenic plants with roots especially root tips showing the highest expression (Fig. 2c). These results indicated the At1g promoter controlled root-preferential expression of At1g::AtGA20ox.

Growth and developmental characterizations of AtGA20ox tobacco plants

Previous studies showed that an overexpression of AtGA20ox increased cell division and elongation and resulted in other morphological alterations in different plant species [14, 19, 28]. In this present study, all AtGA20ox transgenic lines carrying either At1g or 35S promoter exhibited longer stems than WT tobacco. In particular, either At1g-7 and At1g-9 or 35S-2 and 35S-5 showed 1.5-fold increase in the stem length compared to WT plants except that line At1g-5 had about 1.25-fold increase in stem length (Fig. 3b). Both 35S and At1g transgenic tobacco were taller than WT plants. However, stem diameter of 35S transgenic tobacco, particularly 35S-5, was smaller than WT plants. On the other hand, there was no observable difference in stem diameter between At1g::AtGA20ox and WT plants (Fig. 3c). As a result, stem fresh weights of At1g::AtGA20ox lines were greater than 35S::AtGA20ox transgenic lines (Fig. 3d). Of these At1g::AtGA20ox lines, At1g-9 showed the highest increase in stem diameter (~ 110%) and stem fresh weight (~ 200%) compared to WT plants (Fig. 3c, d). Histological analysis of stem cross sections of the 3rd tobacco internode with toluidine blue showed no significant difference in xylem width and cell number between 35S::AtGA20ox transgenic tobacco and WT plants (Fig. 4a). However, there were increases in both xylem width and cell number in certain At1g::AtGA20ox lines (Fig. 3e, f, g). As a result, these At1g::AtGA20ox transgenic tobacco lines had much more stem fresh weight than 35S::AtGA20ox transgenic and WT plants (Fig. 3d). In addition, the 35S::AtGA20ox lines had smaller and curling leaves than WT plants (Fig. 4a). The leaf area of these lines were around 210 cm² in 35S::AtGA20ox transgenic plants as compared to 285 cm² in WT plants (Fig. 4b). Despite this there was no observable difference in leaf area between At1g::AtGA20ox and WT plants. Finally, under greenhouse conditions, all 35S::AtGA20ox transgenic tobacco lines showed a delayed flowering as compared to WT plants (Fig. S4). Their flower buds failed to emerge within 100 days after planting as compared to the flower bud emergence of WT plants approximately 90 days after planting. Interestingly, there was no significant difference in flowering time between At1g::AtGA20ox transgenic tobacco and WT plants (Fig. S4). Together, these results demonstrated the desirable phenotypes of the At1g::AtGA20ox transgenic plants.

Root growth and morphology of transgenic tobacco

All transgenic tobacco plants showed a faster root growth than WT plants (Fig. 5). Of these transgenic plants, At1g::AtGA20ox transgenic lines showed a large increase in the root diameter by up to 2.2-fold (At1g-9) as compared to WT plants. In contrast the root diameter of 35S::AtGA20ox lines was only 1.25-fold increase compared to WT (Fig. 5c). Analysis of root cross section displayed a faster root development in AtGA20ox transgenic plants with bigger xylem zones and higher xylem/phloem ratios than WT (Fig. 5d, e, f). The xylem width of At1g::AtGA20ox roots was from 2- to 2.5-fold increase whereas that of 35S::AtGA20ox roots was 1.2- to 1.5-fold, compared to WT roots (Fig. 5e). The xylem/phloem ratio was from 1.9 to 2.2 in At1g::AtGA20ox and from 1.5 to 1.75 in 35S::AtGA20ox transgenic lines, while that of WT was only 1.45 (Fig. 5f). Consequently, the fresh root weight of At1g::AtGA20ox transgenic tobacco increased from 2 to 4 times compared to 35S::AtGA20ox and WT plants, respectively (Fig. 5b).

Root-preferential expression of At1g::AtGA20ox in woody plant (*M. azedarach*)

Expression of AtGA20ox in transgenic *M. azedarach*

Transgenic *M. azedarach* of At1g::AtGA20ox were generated using *Agrobacterium*-mediated method (Dong et al. 2011) with modifications (Fig. S5). About 250 hypocotyl fragments were used for inoculation and more than 30 transgenic lines were produced on selection medium. We randomly selected 13 lines to confirm the presence of AtGA20ox gene. Of these, 12 plants showed the expected PCR bands in gel run indicating the integration of AtGA20ox gene in *M. azedarach* genome (Fig. 6a). Semi-quantitative RT-PCR and gel-run were performed using GADPH gene as an internal control to evaluate AtGA20ox transcript level in transgenic *M. azedarach* (Fig. 6b). All tested plants showed transcript levels of AtGA20ox gene compared to WT plants. In addition, the expression levels of AtGA20ox gene were higher in root than in the leaf and stem of all *M. azedarach* transgenic lines. Different tissues of transgenic line At1g-1 including leaves, stems, roots and root tips were also analyzed by quantitative real-time-PCR to examine the expression patterns of AtGA20ox gene which were highly consistent with those in At1g::AtGA20ox transgenic tobacco. The higher expression level of AtGA20ox was in root tissue, especially root tips (Fig. 6c), which was as high as 1.4- and 2-fold compared to that in root elongation zone and young stem. The AtGA20ox expression in leaves was much lower than other tissues. Together, these results again indicated the preferential-root expression of At1g promoter.

Increased stem and root growth

At three months under greenhouse conditions, all transgenic plants exhibited a faster growth than WT plants (Fig. 7). The stem length increased two-fold in transgenic plants compared to WT plants (Fig. 7c). In addition, the stem diameter was greater in all At1g::AtGA20ox transgenic *M. azedarach* (Fig. 7d). Stem cross-sectional analysis showed a large xylem zone and more xylem cell number in transgenic *M. azedarach* than in WT plants. As a result, transgenic lines At1g-1, At1g-5 and At1g-14 displayed around two-fold increase in the stem fresh weight as compared to WT plants. This result was highly consistent with that of transgenic AtGA20ox tobacco.

Similar to the observations on At1g::AtGA20ox tobacco, all transgenic *M. azedarach* exhibited a faster root growth and bigger root system than WT plants (Fig. 8). The bigger xylem zone and more numbers of xylem cells were observed in all transgenic plants. The xylem zone of transgenic plants varied from 668 μm (At1g-14) to 706 μm (At1g-5), while that for WT plants was around 524 μm . The xylem cell number increased from 32 in WT plants to up to 44 in At1g::AtGA20ox plants (Fig. 8e, f). Furthermore, the xylem/phloem ratio was much higher in transgenic lines than WT plants (Fig. 8d, g). Importantly, transgenic *M. azedarach* plants showed over two-fold increase in root fresh weight compared to WT (Fig. 8b). The highest root fresh weight was obtained in transgenic line At1g-5 which had the largest xylem zone and the highest xylem cell number. Consequently, all transgenic plants had a much higher dry root weight (Fig. 8c). These results demonstrated a great utility of At1g::AtGA20ox root-preferential expression for root growth and root biomass production of *M. azedarach* and potentially other woody plants.

Discussions

To overcome various undesirable limitations of using a constitutive promoter, the employment of a tissue-specific promoter instead has become an approach of choice and achieved more desirable results. Jeon and colleagues used the DX15 promoter (xylem tissue-specific promoter) to drive PdGA20ox1 gene expression in transgenic poplar plants, which enhanced plant growth with the partial elimination of the unwanted phenotype [22]. However, leaf area and root mass of these transgenic plants were still lower than those of WT plants. In addition, other studies using root-specific promoter for AtGA20ox overexpression demonstrated the superiority over the constitutive promoters [29–31]. In agreement with previous reports, the At1g::AtGA20ox transgenic plants showed enhanced plant growth and root development without comprising leaf area and flowering time compared to WT. Therefore, our results here illustrated the superiority of using tissue-preferential promoter instead of constitutive promoter in overexpressing AtGA20ox for plant biomass improvement.

Prior to our study, Vijaybhaskar and colleagues indicated that At1g promoter was a root-specific promoter based on GUS assay in *Arabidopsis* [27]. However, no transgene expression at transcription levels under the control of At1g promoter had been characterized publicly before. In our research, we visually observed GUS activity only in root tips of At1g::AtGA20ox transgenic tobacco instead of the entire root, not as reported by Vijaybhaskar et al. [27]. This difference in GUS expression may be due to the differences in promoter activities from plant to plant. For example, the rolD promoter was described as a root-specific promoter in tobacco [32]. Nevertheless, the promoter showed a strong expression in both roots and shoots of pea and *Gladiolus* plants [33, 34]. In the report by Vaughan et al., the FaRB7 promoter showed root-specific expression in strawberry but constitutive expression in tobacco plants [35]. In our work, the qRT-PCR results exhibited the expression throughout the entire plant of AtGA20ox under the control of At1g promoter (Fig. 6c). In these transgenic plants, the transcription levels were in the descending order of root tips, root elongation zones, stems and leaves. This result agrees with that of Chen et al. who used a “root-specific” promoter GmPrP2 for soybean transgenic plants [29]. Their qRT-PCR results showed that the transgene was not only expressed strongly in roots, but also in stems, leaves, flowers, seeds and

hypocotyls, suggesting a root-preferential rather than root-specific expression. A more recent study reported that At1g was a root-specific promoter in Arabidopsis, mainly based on GUS assay [27]. However, the transcription analysis, which is more sensitive than reporter gene analysis, has not been conducted in their study. Here, we have demonstrated that At1g is a root-preferential promoter with its expression level varying in different plant tissues. Thus, our study has provided more insightful information about the At1g promoter function employing not only reporter gene but also the gene of interest.

Previously, different root-specific promoters had been isolated and analyzed for spatiotemporal expression using reporter genes [27, 29, 32, 34, 36]. Jeong and colleagues utilized the root-specific promoter Rcc3 to drive OSNAC10 gene and observed a strong gene expression in root as well as enhanced plant growth [37]. Moreover, the root-specific expression of this gene improved drought tolerance of transgenic rice during reproductive stage and increased yield by 10–20% under both normal and drought conditions under greenhouse condition. However, very few researches later observed the effect of root-specific expression of their target genes on the phenotype, growth and development of transgenic lines as Jeong et al. reported [37]. Most publications on root-specific promoters have been confined to the analysis of promoter expression through the GUS gene [27, 29, 34, 36, 38]. In our study, we evaluated AtGA20ox expressions and functions under the control of At1g promoter. The gene expression profile was highly correlated to the plant morphology and biomass production which included the enhanced stem and root growth and improved biomass production. The desirable phenotypes observed in both tobacco and *M. azedarach* demonstrated the great potential of this promoter for the gene preferential expression in root tissue for plant biomass improvement. Further researches are needed to evaluate the effects of At1g::AtGA20ox root-preferential expression on drought tolerance of transgenic plants.

Conclusions

Our results showed that At1g promoter controlled the strong expression of AtGA20ox target gene in roots. The root system of transgenic lines developed much stronger than the control lines, in terms of both volume and weight. Stems and leaves also exhibited AtGA20ox gene, but in lesser degree. This minimizes the undesirable phenotype of strong expression of target genes in all parts of the transgenic lines with the 35S promoter. At1g promoter has great utility in genetic engineering to improve growth and biomass or enhance stress tolerance in woody plants and potentially some other plant species.

Methods

Plant materials and growth conditions

The in vitro plants of *Nicotiana tabacum* cultivar K326 were generated in Institute of Biotechnology, Vietnam Academy of Science and Technology. Mature seeds of *M. azedarach* L. were supplied by Vietnam National University of Forestry. Plants were grown under greenhouse conditions (27 ± 2 °C, 70% of humidity, 14 photoperiod, $350 \mu\text{mol m}^{-2} \text{s}^{-1}$) using mixed soil (1 Tribat (Saigon Xanh, Vietnam): 1 TN1

(Research Center for Fertilizers and Plant Nutrients, Vietnam)) in the plastic pot (E280 × 260) for tobacco and FAA530 (450 × 300 × 300) for *M. azedarach*.

Vector construction

The specific forward and reverse primers (At1gF/At1gR) were designed based on the sequence of At1g73160 Arabidopsis promoter [27] (Table S1) and used to amplify this promoter from Arabidopsis genomic DNA. AtGA20ox gene was amplified from Arabidopsis thaliana genomic DNA using primer pairs specific to this gene sequence [26] (Table S1). The amplified AtGA20ox gene and At1g promoter were confirmed by Sanger sequencing and inserted into the binary vector pBI121. To do so the 35S promoter region of the pBI121 was replaced with At1g promoter as a HindIII/SmaI fragment while the GUS gene was replaced by the AtGA20ox gene, resulting in the transformation construct pBI121/At1g::AtGA20ox vector (Fig. S2h, i, k).

Tobacco and *M. azedarach* transformation

Leaves of tobacco in vitro seedlings were used as explants for Agrobacterium-mediated transformation following protocol of Topping (1998) with modifications [39]. Briefly, single *A. tumefaciens* C58 colony carrying the transformation constructs was cultured on the LB medium [40] supplemented with appropriate antibiotics and kept at 28 °C, 250 rpm until the bacterial density reached the OD₆₅₀ of 0.6. The bacterial cells were harvested by centrifugation at 5,000 rpm at room temperature for 10 min and then suspended in ½ MS liquid medium containing 200 µM acetosyringone (Sigma-Aldrich, Inc) (inoculation medium). The in vitro tobacco leaf disks (1 × 1 cm²) were prepared and soaked in bacterial suspension with gentle agitation for 30 min. The infected leaf disks were placed on the co-cultivation media (CCM-K) at 25 ± 2 °C in dark for 2 days. After co-cultivation, the leaf disks were washed with sterile water, blotted by filter papers and cultured on selection medium (SM-K) containing 150 mg/L kanamycin. Healthy shoots formed from the leaf disks were transferred to root induction medium (RM-K). Rooted plants were moved to mixed soil under greenhouse conditions.

For *M. azedarach* transformation, sterilized seeds were germinated on MS medium [41] containing 3% (w/v) sucrose and 7.5 g/L agar in 250 ml glass flasks. The cultures were kept in dark at 24 ± 1 °C for 2 days and then transferred to 14 h photoperiod. After 2 weeks on seed germination medium, the hypocotyl was cut into 1 cm fragments and used for Agrobacterium-mediated transformation following the procedure described by Dong et al. [42].

Confirmation of transgene integration and expression

Genomic DNA was isolated from young leaves following CTAB method [43] and was used for PCR reactions using specific forward and reverse primers GA20ox-F/R (Table S1) to confirm integrated transgenes. The PCR reactions were performed as follows: Initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 50 s, followed by final extension at 72°C for 7 min. Then, PCR products were analyzed by electrophoresis using 0.8% agarose gel.

To confirm transgene expression, total RNA was extracted from roots, stems and leaves of 45-day-old tobacco and 90-day-old *M. azedarach* plants using TRIzol™ Reagent following manufacture's protocol (Thermo Fisher Scientific-Promega, Madison, WI, USA). The RevertAid First Strand cDNA synthesis Kit (Thermo Fisher Scientific) was used to synthesize the cDNA from the total isolated RNA. Semi-quantitative RT-PCR was performed to analyze the expression of AtGA20ox gene using *Nicotiana tabacum* Actin or *M. azedarach* GADPH gene as an internal control. qRT-PCR was carried out utilizing Advanced TM Universal SYBR® Green Supermix. The primers for RT-PCR and qRT-PCR were given in Table S1. Gene expression levels were normalized and analyzed using three clonal plants of each events as biological replicates.

Plant growth measurements

At1g::AtGA20ox transgenic and wild-type (WT) plants were transferred to pots of mixed soil under greenhouse conditions and stem length was measured every 5 days for tobacco and 15 days for *M. azedarach* plants. Other morphological characteristics such as stem and root fresh weights, stem diameters, leaf areas were also measured from tobacco 45 days after being transferred to greenhouse. For *M. azedarach*, the measurements were taken 90 days after being transferred to greenhouse.

Histological analysis

Stem and root cross sections of tobacco and *M. azedarach* plants were collected and histologically analyzed. The cross sections were stained with 2% phloroglucinol/HCl or 0.05% toluidine blue for 1 min. Images were observed and captured by Meiji Techno MA151/MT05 C-mount 0.5 × Microscope (at 4×, 10 × and 40×). Xylem and phloem width were identified from the images using method described by Jeon et al. [22].

Data analysis

The complete random design was applied to the experimental units under tissue culture and greenhouse conditions. All treatments were conducted in at least three replicates. Data were collected and analyzed by SPSS software Version 20 (Chicago, IL) and Microsoft excel program.

Additional file1: Figure S1. Diagram of T-DNA regions of transgene constructs used in this study. Figure S2. Construction of vector pBI121/At1g::AtGA20ox. (a) Amplification of At1g fragment from total DNA of *A. thaliana* using At1gF/R primers. (b) Amplification of At1g fragment from white colonies in blue white screening using M13F/R primers. (c) Treatment pBT/At1g and pBI121 by HindIII and SmaI. (d) Amplification of At1g fragment from *E. coli* DH5α. (e) Treatment pBI121/At1g by HindIII and SacI. (f) Amplification of At1g fragment from *A. tumefaciens* C58. (g) Treatment pBI121/At1g::GUS and pBT/GA20ox by SmaI and SacI. (h) Amplification of GA20ox gene from *E. coli* DH5α. (i) Treatment pBI121/At1g::GA20ox by BamHI and SacI. (j) Amplification of At1g and GA20ox from *A. tumefaciens* C58. M. GeneRuler™ 1 kb DNA Ladder; 1–10. Colony lines. Figure S3. Tobacco transformation using the pBI121/At1g::AtGA20ox construct. Figure S4. Flowering time of tobacco plants at 110 days under greenhouse condition. Student's t-test showing significance as compared with WT at *P < 0.05 (n = 5).

Figure S5. *Melia azedarach* transformation using pBI121/At1g::AtGA20ox construct. Table S1. The primers used in this research

Abbreviations

+ and -: Positive and negative control, respectively; A and S: transgenic plants generated from At1g::AtGA20ox and 35S::AtGA20ox constructs, respectively; At1g::GUS and 35S::GUS: transgenic tobacco with GUS gene guided by At1g promoter and 35S promoter, respectively; At1g pro: *A. thaliana* 1 glycosyltransferase 73160 promoter; CCM: co-cultivation medium; GA20ox: Gibberellin (GA) 20-oxidase gene; GUS: β -1,4-Glucuronidase gene; 35S pro: 35S promoter; LB: left border; NeoR/KanR: Neomycin phosphotransferase II gene; NOS pro: Nopaline synthase promoter; NOS ter: Nopaline synthase terminator; RB: right border; RM: root formation from shoots on rooting medium; SM: seed-derived seedlings on MS medium; SM1, SM2: shoot induction on the first and second selective medium containing kanamycin, respectively; T0-1: a representative putative transgenic line in soil; WT: non-transgenic plants

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All the data used in this study are mentioned within the article and its additional files.

Competing interests

The authors declare no competing financial interest.

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Authors' contributions

LL, DP and CH designed the study; CH and PN conceived and supervised the study; LL and BT conducted the experiments; LA provided initial training and assisted histological analysis; NP assisted the

experiments; LL and BT analyzed data; LL and DP wrote manuscript; ZZ and CH revised and proof-read the manuscript. All authors have read and approved the manuscript.

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Figures

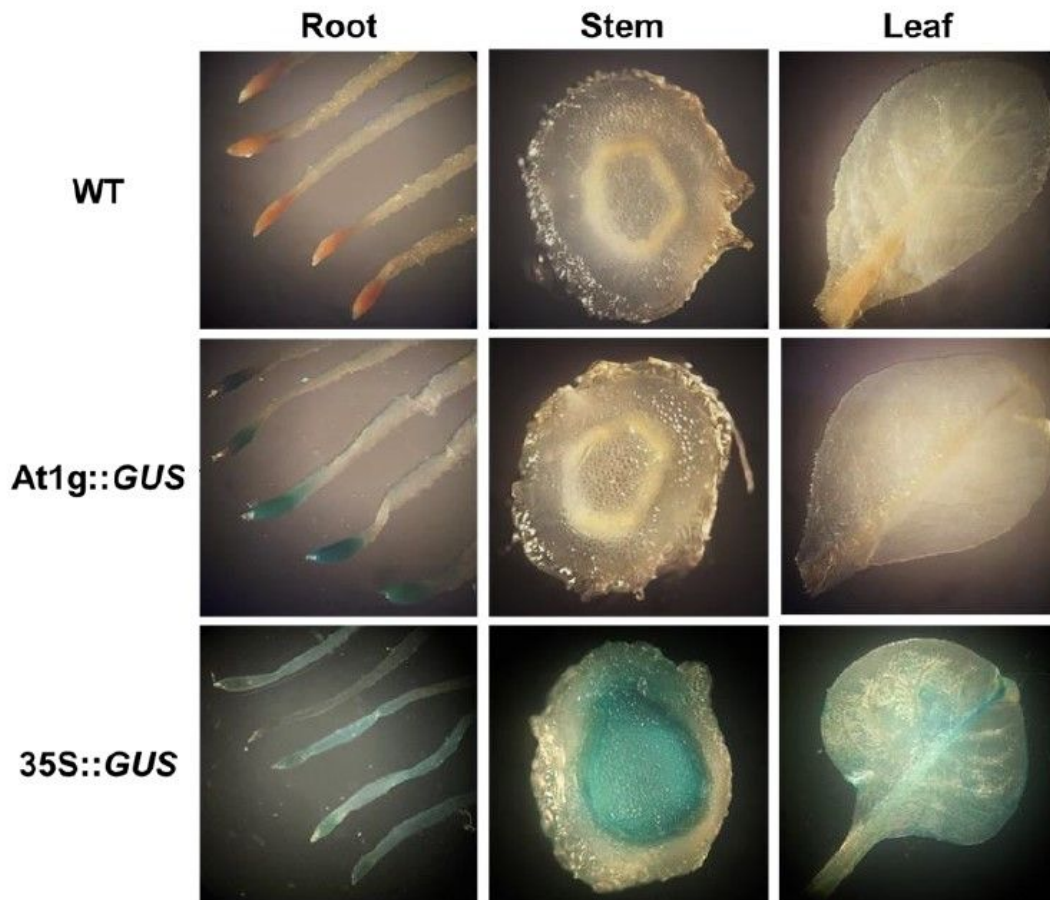


Figure 1

Expression patterns of GUS gene in different plant tissues.

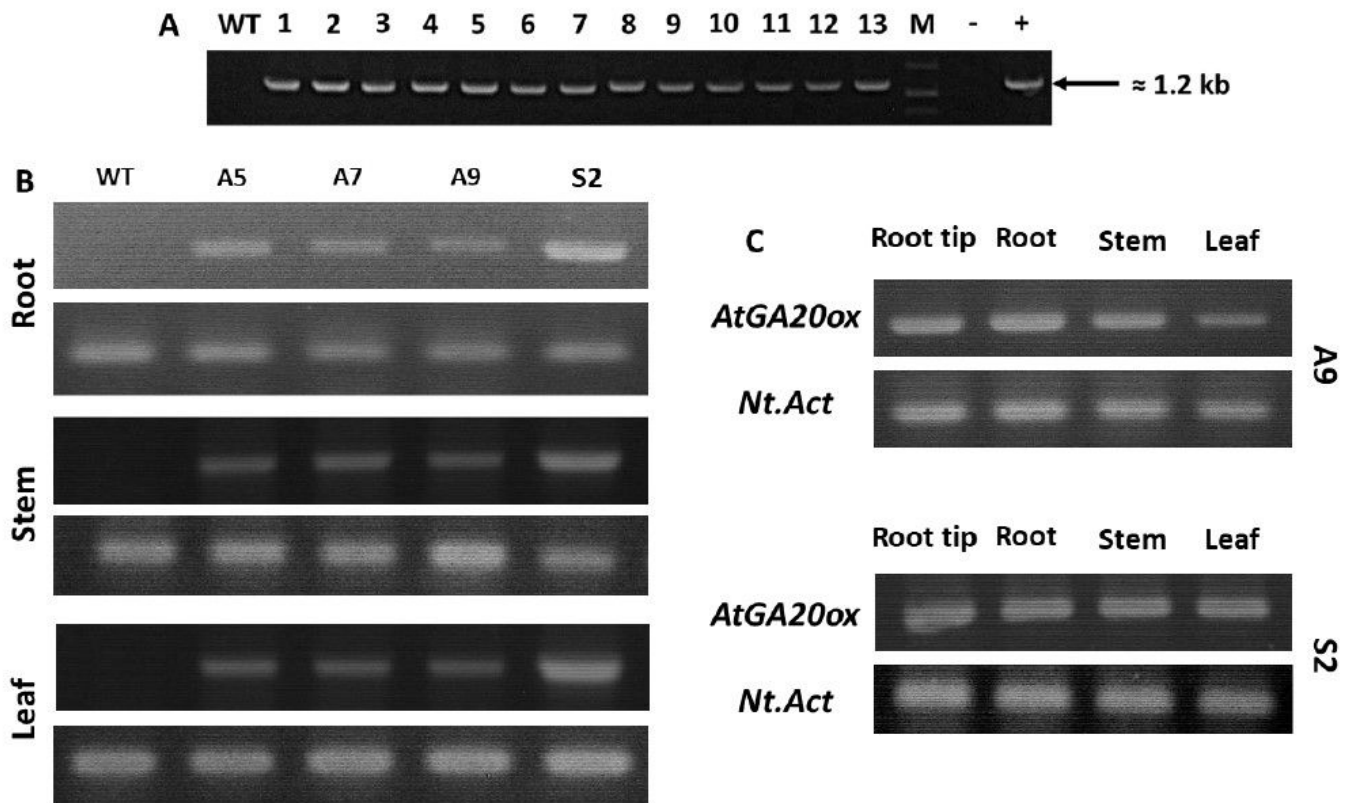


Figure 2

Presence and expression of *AtGA20ox* in transgenic tobacco. a PCR-gel running using specific primers for *AtGA20ox* gene. b, c RT-PCR of *AtGA20ox* gene (above) and Actin gene (bottom) in different tissues of transgenic tobacco, respectively; Lanes 1-13, different transgenic lines.

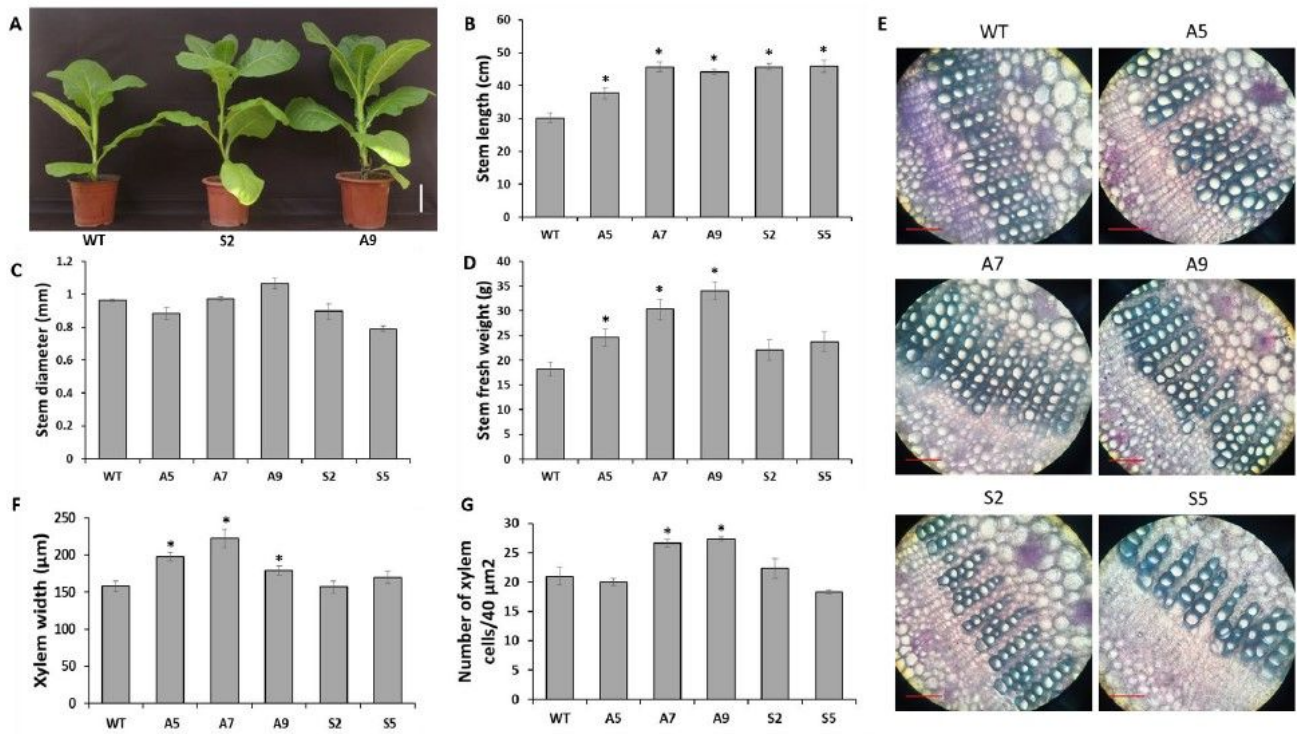


Figure 3

Transgenic tobacco phenotypes under greenhouse conditions. a Tobacco plants at 45 days under greenhouse conditions. Scale bar = 10 cm. b, c, d Stem length and diameter as well as fresh weight of tobacco plants. e Stem cross sections stained by toluidine blue. Scale bar = 100 μm . f Xylem width of stem cross sections. g Number of stem xylem cells per 40 μm^2 . Student's t-test showing significance as compared with WT at * $P < 0.05$ ($n=5$).

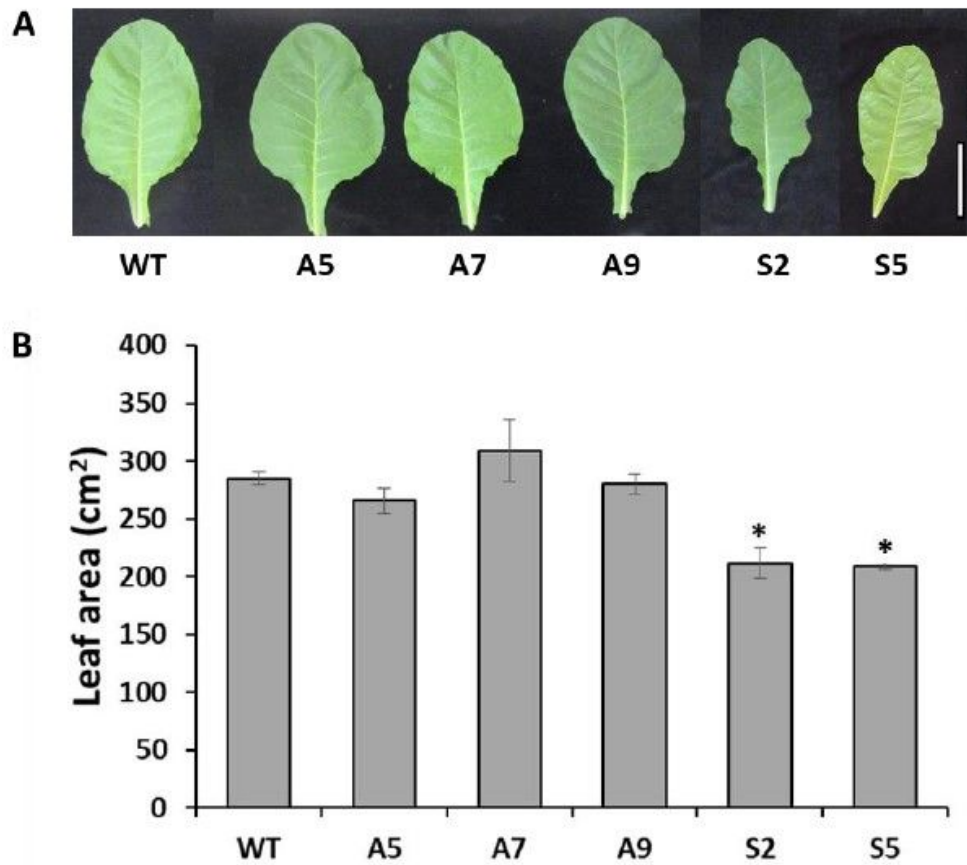


Figure 4

Leaf morphology of overexpressed AtGA20ox tobacco. a Leaf shapes and sizes. Scale bar = 10 cm. b Leaf area of tobacco plants. Student's t-test showing significance as compared with WT at *P < 0.05 (n=5).

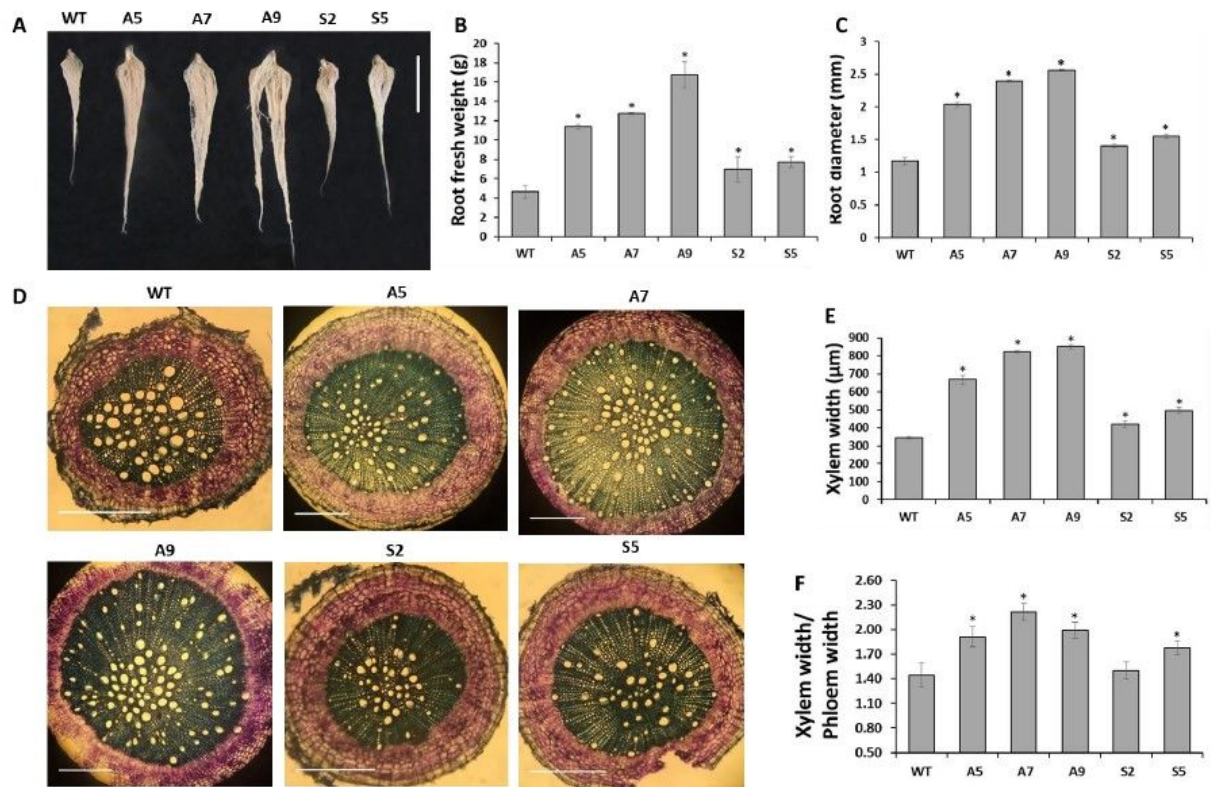


Figure 5

Root phenotype and morphology of transgenic tobacco. a Root systems of tobacco under greenhouse conditions. Scale bar = 10 cm. b Root fresh weight. c Root diameters. d Root cross sections stained by toluidine blue. Scale bar = 500 μm. e Xylem width of root cross sections. f Ratio of xylem over phloem zones. Student's t-test showing significance as compared with WT at *P < 0.05 (n=5).

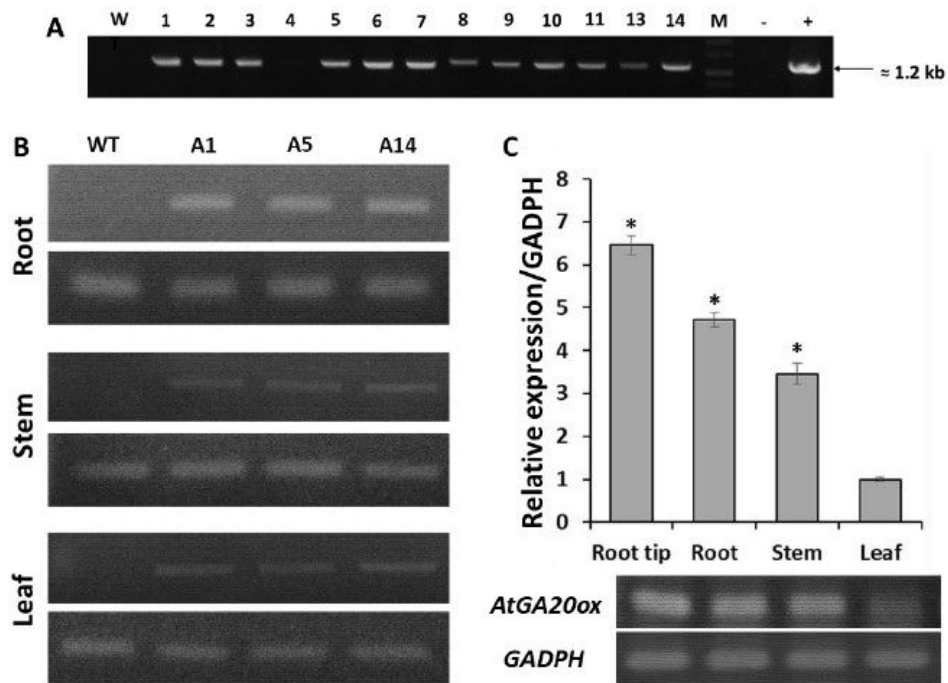


Figure 6

Presence and expression of AtGA20ox in transgenic *Melia azedarach*. a PCR-gel running using specific primers for AtGA20ox gene. b RT-PCR of AtGA20ox gene (above) and GADPH gene (bottom) in different tissue of transgenic plants; Lanes 1-13, different transgenic lines. c Realtime-qPCR (above) and RT-PCR (bottom) of AtGA20ox and GADPH genes in different tissues of transgenic plants line 1.

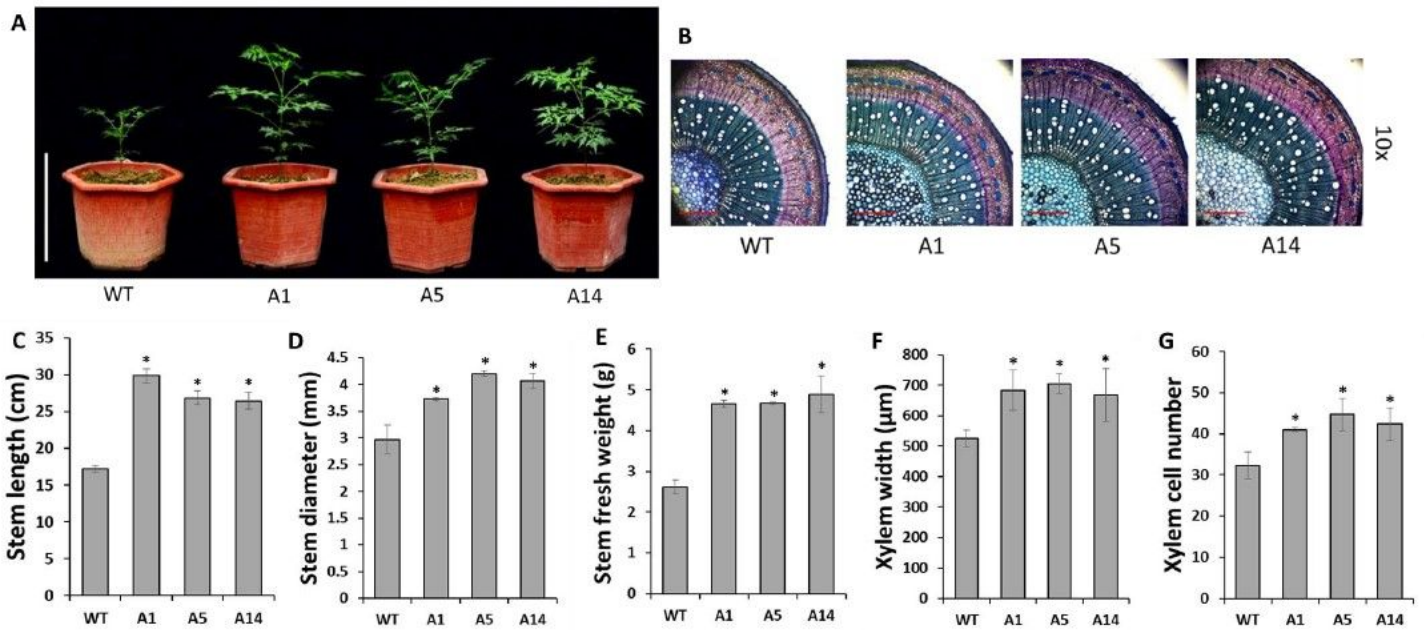


Figure 7

Plant morphology of transgenic *Melia azedarach*. a *M. azedarach* at 3 months under greenhouse conditions. Scale bar = 20 cm. b Stem cross sections stained by toluidine blue. Scale bar = 500 μm. c Stem length. d Stem diameter at 10 cm from the ground. e Stem biomass. f Xylem width of stem cross sections. g Number of xylem cell per pile. Student's t-test showing significance as compared with WT at *P < 0.05 (n=5).

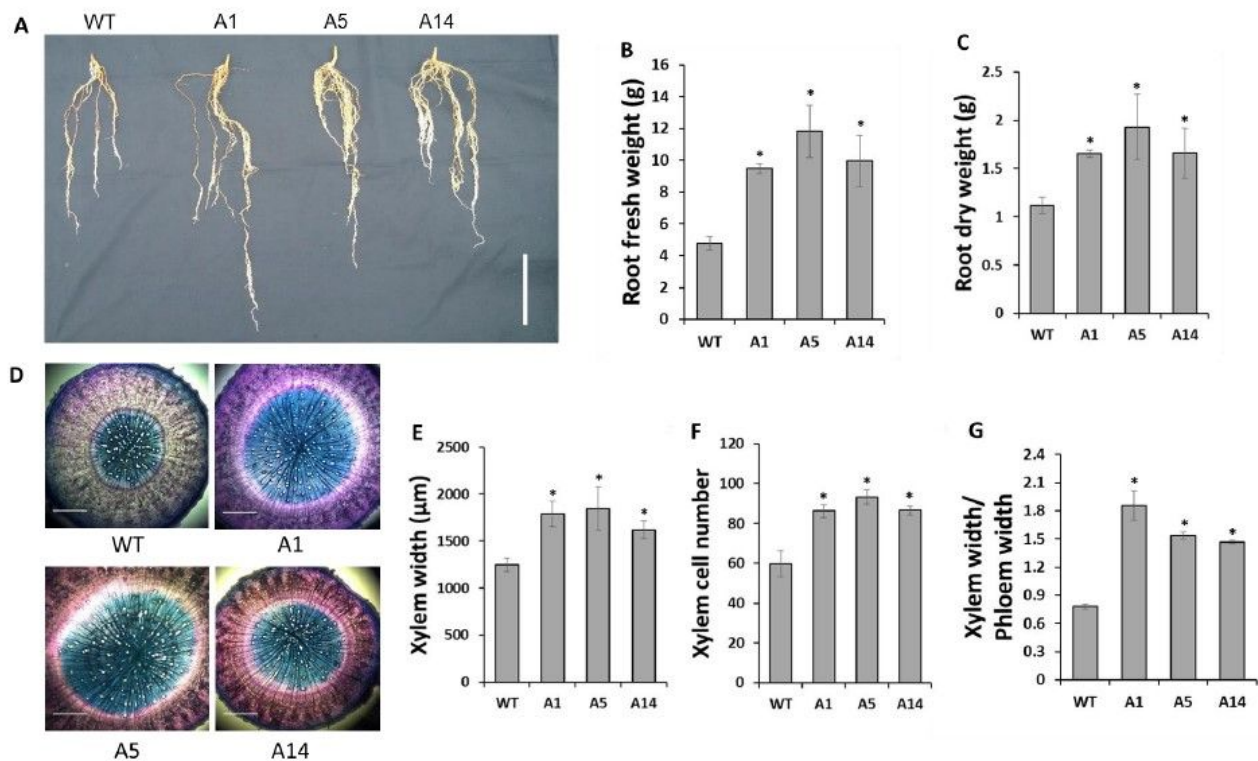


Figure 8

Root morphology of transgenic *Melia azedarach*. a *M. azedarach* root system under greenhouse conditions. Scale bar = 20 cm. b and c Root fresh and dry weight, respectively. d Root cross sections stained by toluidine blue. Scale bar = 1000 μm . e Xylem width of root cross sections. f Number of xylem cell per pile. g Ratio between xylem and phloem zones of root cross sections. Student's t-test showing significance as compared with WT at $*P < 0.05$ (n=5).

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