Introduction of the rice *CYP714D1* gene into *Populus* inhibits expression of its homologous genes and promotes growth, biomass production and xylem fibre length in transgenic trees

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Abstract

The rice (*Oryza sativa*) OsCYP714D1 gene (also known as *EUI*) encodes a cytochrome P450 monooxygenase which functions as a gibberellin (GA)-deactivating enzyme, catalysing 16α, 17-epoxidation of non-13-hydroxylated GAs. To understand whether it would also reduce the production of active GAs and depress the growth rate in transgenic trees, we constitutively expressed OsCYP714D1 in the aspen hybrid clone *Populus alba* × *P. berolinensis*. Unexpectedly, ectopic expression of OsCYP714D1 in aspen positively regulated the biosynthesis of GAs, including the active GA$_{1}$ and GA$_{4}$, leading to promotion of the growth rate and biomass production in transgenic plants. Transgenic lines which showed significant expression of the introduced OsCYP714D1 gene accumulated a higher GA level and produced more numerous and longer xylem fibres than did the wild-type plants. Quantitative real-time PCR indicated that transcription of most homologous PtCYP714 genes was suppressed in these transgenic lines. Therefore, the promoted GA and biomass production in transgenic trees constitutively expressing OsCYP714D1 is probably attributed to the down-regulated expression of the native PtCYP714 homologues involved in the GA biosynthesis pathway, although their precise functions are yet to be further elucidated.

Key words: Aspen, biomass, gibberellin, OsCYP714D1, *Populus*, transgenic plants.

Introduction

In plants, gibberellins (GAs) play crucial roles in shoot elongation and reproductive development, including stem elongation, leaf expansion, seed germination, and flowering. Most genes involved in the biosynthesis and catabolism pathways of GAs have been identified, especially in the model plant species *Arabidopsis thaliana* and rice (*Oryza sativa*) (Hedden and Phillips, 2000; Olszewski et al., 2002; Sun and Gubler, 2004). Also, many mutants with altered GA metabolism or signalling pathways have been studied in *A. thaliana* (Koornneef and van der Veen, 1980; Vabanova et al., 2007; Ariizumi et al., 2008) and rice (Ueguchi-Tanaka et al., 2000; Ikeda et al., 2001; Sasaki et al., 2003; Sakamoto et al., 2004). These GA-related mutants have been used to identify the key components in GA metabolism and signalling pathways, and some of them have successfully contributed to the green revolution to confer useful agronomic traits in cereals (Peng et al., 1999; Sasaki et al., 2002; Spielmeyer et al., 2002). Previous studies have shown that GAs are biosynthesized from geranylgeranyl diphasphate, and three types of enzymes, plastid-localized terpene cyclases, membrane-bound...
cytochrome P450 monoxygenases (P450s), and soluble 2-oxoglutarate-dependent dioxygenases (2ODDs), are responsible for the conversion of geranylgeranyl diphosphate into bioactive GAs, such as GA\textsubscript{1} and GA\textsubscript{4} (Zhu et al., 2006). The bioactive GA\textsubscript{1} and GA\textsubscript{4}, and their immediate precursors GA\textsubscript{20} and GA\textsubscript{24}, respectively, are further deactivated by GA 2-oxidases (GA2oxs) (Thomas et al., 1999; Yamaguchi and Kamiya, 2000; Olszewski et al., 2002). In Arabidopsis, AtGA2ox7 and AtGA2ox8 also catalyse the 2-oxidation of C\textsubscript{20}GAs (Schomburg et al., 2003; Lee and Zeevaart, 2005).

To date, a few loss-of-function mutants of the GA2ox gene family members have been recognized (Hedden and Phillips, 2000; Sakamoto et al., 2004). In pea (Pisum sativum), the loss-of-function mutation in the PsGA2ox1 gene causes a tall phenotype (Lester et al., 1999). In rice, the recessive tall rice mutant elongated uppermost internode (euI) (Rutger and Carnahan, 1981), which demonstrates a rapid and enhanced elongation of internodes, has been used to improve the headling performance of male sterile cultivars genetically (Shen and He, 1989; He and Shen, 1991, 1994; Yang et al., 2002). Recently, the EUI gene was isolated. Map-based cloning reveals that EUI encodes a previously uncharacterized P450, CYP714D1, that acts as a GA-deactivating enzyme through 16α, 17-epoxidation of 13-hydroxylated GAs (Zhu et al., 2006).

In rice, 16α, 17-(OH)\textsubscript{2}-GA\textsubscript{4} has been identified as a metabolite of GA\textsubscript{4} exogenously applied to seedlings (Kobayashi et al., 1993), and as an aglycone of a GA glucoside in anthers (Hasegawa et al., 1994). The 16α, 17-(OH)\textsubscript{2}-GAs were also found in other plant species, such as Pisum sativum fruits (Santos et al., 1995), Lupinus albus seeds (Gaskin et al., 1992), developing Malus domestica seeds (Hedden et al., 1993), Cibotiumglaucum sporophytes (Yamane et al., 1988), Prunus avium seedlings (Blake et al., 2000), and Populus trichocarpa capsules (Pearce et al., 2002). However, whether these 16α, 17-(OH)\textsubscript{2}-GAs are produced via 16α, 17-epoxy GAs by EUI-related enzymes is still unclear in these plant species, especially in the model woody plant Populus trichocarpa. In this work, the rice OsCYP714D1 gene was constitutively expressed in aspen. It was found that ectopic expression of OsCYP714D1 in transgenic aspen positively regulated the biosynthesis of GAs, leading to promotion of the growth rate and biomass production. Moreover, transcription of most homologous PtCYP714 genes was suppressed in the fast-growing transgenic lines. These findings will aid in future attempts to engineer the growth trait by manipulating biosynthesis of GAs in aspen, possibly as well as in other plants.

Materials and methods

Plasmid construct and plant transformation

The plant vector was generously provided by Professor Zuhua He (Shanghai Institutes for Biological Sciences, CAS, China). The 1.9kb rice OsCYP714D1 (OsEUI) full-length cDNA, driven by the Cauliflower mosaic virus (CaMV) 35S promoter and terminated by the NOS terminator, was inserted into the EcoRI and HindIII sites in pCAMBIA1301 (http://www.cambia.org) (Zhu et al., 2006). The construct was introduced into the Agrobacterium tumefaciens strain EHA105 using the freeze–thaw method (Wise et al., 2006) for aspen transformation.

In this study, the Yinzhong (P. alba × P. berolinensis) hybrid clone, which is commercially grown in the north part of China, was used for plant transformation. Generally, in vitro grown plants were subcultured monthly by aseptically transferring shoot apices to fresh MS medium (Murashige and Skoog, 1962) supplemented with 0.1 mg l\textsuperscript{-1} naphthaleneacetic acid (NAA). Plantlets were grown in the tissue culture room with cool white fluorescent light (~200 μmol m\textsuperscript{-2} s\textsuperscript{-1}) under short day conditions (12 h light/12 h dark). The temperature was kept at 21–25°C in the day time and at 15–18°C at night. The OsCYP714D1 gene was transformed into the hybrid aspen as described previously (Wang et al., 2011). Independently regenerated transgenic lines were propagated, potted, and grown in the greenhouse.

PCR, reverse transcription–PCR (RT–PCR), and quantitative real-time PCR

For PCR analyses, genomic DNA was isolated from fresh leaves (~500 mg for each sample) of 1-month-old tissue cultured wild-type (WT) and regenerated transgenic lines as described previously (Kang et al., 2010). Gene-specific primers (forward, 5’-CCGGGCTTGCTTTGGGAGTGA-3’; and reverse, 5’-CCGCCGCAGACCTCGAGACCT-3’) and GC buffer (TaKaRa, Japan) were used to amplify a 463 bp PCR product.

For RT–PCR analyses, total RNA was isolated from shoots of 1-month-old tissue cultured WT and transgenic plants with the RNAsio Reagent (TaKaRa, Japan). After treatment with DNase I (Promega), 2 μg of total RNA was subjected to reverse transcription using the reverse transcriptase ReverTra Ace (TaKaRa, Japan), and the expression value of the WT was set to 1. The primers used in this research are shown in Supplementary Table S1 available at JXB online.

β-Glucuronidase (GUS) activity analysis

Histochemical GUS staining was conducted as described previously (Gallagher, 1992). Briefly, leaf explants were incubated overnight at 37 °C in a reagent mix containing 2 mM 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-Gluc), 0.1 mM sodium phosphate buffer (pH 7.0), 0.5 mM each of potassium ferri- and ferrocyanide, 10 mM EDTA (pH 7.0), and 0.1% Triton X-100. After staining, the tissues were cleared of chlorophyll with 95% ethanol.

Southern blot analysis

For Southern blot analysis, 10 μg of genomic DNA isolated from the leaves of WT and transgenic plants was digested with EcoRI, electrophoresed on 0.8% agarose gels, and transferred onto Hybond N* nylon membranes with a Whatman Biomera® Vacuum-Blot System. The 463 bp PCR product of OsCYP714D1 was used as a hybridization probe. Standard procedures for Southern blot analysis and probe labelling were conducted with a DIG DNA Labeling and Detection Kit1 (Roche, Germany) following the manufacturer’s instructions.
Growth measurement and sample collection

Rooted WT and transgenic plants were transplanted into soil and maintained in the greenhouse. Plants were watered every 2 d and fertilized with a complete nutrient solution once a week. After 2 weeks, healthy plants (at least eight individuals for each line) were marked at the base internode. This was used as a reference point for measuring the growth of plants. The height was counted from the top to the reference point every week for a period of 6 weeks. The number of leaves and internodes of 8-week-old plants were counted, with the first internode being defined as that below the first leaf of at least 1 cm length in the apex. The average of internodes (10th, 11th, and 12th) with the upper leaves was used to measure the diameter of stems, and the length of internodes and petioles, respectively.

For GA content analysis, the upper leaves of the 11th internode were sampled and immediately frozen in liquid nitrogen. For anatomical studies, the 11th internode was excised and immediately fixed in FAA solution. The 18th internode was excised and chilled on ice immediately for subsequent fibre length measurements. All of the remaining part after sampling was separated into shoot (including stem and leaves) and root fractions, and used for determination of the fresh weight, and then, after drying at 65 °C for 5 d, for dry weight determination.

Anatomical characterization

Following conventional chemical fixation in FAA and dehydration in an ethanol series, samples (the 11th internode of each line) were embedded in paraffin. Transverse or longitudinal sections of 8 μm thickness were cut on a microtome (Leica, Germany). The paraffin-embedded sections were dried for 2 d at 42 °C and then dewaxed and re-hydrated as follows. The sections were immersed in dimethylbenzene for 5–10min and this was repeated once with fresh dimethylbenzene. Then, the sections were immersed in 50% dimethylbenzene (diluted in absolute ethyl alcohol) followed by a gradient of ethyl alcohol (100, 95, 85, and 70%) to re-hydrate. For all of the solutions, the immersion time was 5–10min. Following that, the sections were immediately dipped into 0.2% toluidine blue (0.2 g of toluidine blue dissolved in 60 ml of ethyl alcohol and 40 ml of distilled water) for 10 s and then washed in water twice (5–10 s each time). The dyed sections were used for microexamination. Images were captured under bright field using an ECLIPSE 80i microscope. Using the UTHSCSA Image Tool software, the radial type differences were observed between WT and transgenic lines (L4, L6, L13, L23, L29, L32, and L33) were identified by PCR analyses. The expected 463 bp band was detected in all the checked transgenic lines (Fig. 1B). RT-PCR and GUS staining analyses further confirmed the expression of OsCYP714D1 and GUS in transgenic plants (Fig. 1C, D). Transgenic plants were subsequently transferred to pots and grown in the greenhouse for further experiments.

Fibre length measurements

For fibre length measurements, trimmed pieces of outer xylem from the 18th internode of 2-month-old plants grown in the greenhouse were prepared. The samples were macerated in a boiling solution of 10% hydrogen peroxide and 50% glacial acetic acid for 4–6 h, rinsed with distilled water three times, neutralized with sodium carbonate, and washed again in water. Finally, the fibres were separated from each other in water, and measured under an ECLIPSE 80i microscope. The lengths of at least 300 fibres per sample were measured.

GA and IAA content determination

Sampled leaves were homogenized in liquid nitrogen using a mortar and pestle, and then lyophilized. Samples of 2 g dry weight (DW) were purified and finally analysed at Wuhan University (China) as described previously (Chen et al., 2011). \([\text{H}_3]^\text{GA}_{1}\) (1 ng g\(^{-1}\)), \([\text{H}_2]^\text{GA}_4\) (10 ng g\(^{-1}\)), \([\text{H}_2]^\text{GA}_5\) (10 ng g\(^{-1}\)), \([\text{H}_2]^\text{GA}_{12}\) (10 ng g\(^{-1}\)), \([\text{H}_2]^\text{GA}_{15}\) (30 ng g\(^{-1}\)), \([\text{H}_2]^\text{GA}_{33}\) (50 ng g\(^{-1}\)), and \([\text{H}_2]^\text{indole acetic acid (IAA; 10 ng g}^{-1}\)) were added to plant samples as internal standards and the recovery was 87.1–108.6%. Except for GA\(_1\), which was detected at low concentration in three transgenic lines L4, L13, and L32, but not detected in the WT and line L23, significant differences in IAA, GA\(_12\), GA\(_5\), GA\(_6\) between transgenic plants and the WT were analysed using Student’s t-test at \(P < 0.05\). Values are means ±SD of three biological replicates of three individual plants from the WT or the same transgenic line.

Statistical analysis

For statistical analyses, the Student’s t-test was used to generate every \(P\)-value. The tests were one-tailed. The data were normalized and all samples were normally distributed with homogeneity of variance.

Results

Constitutive expression of OsCYP714D1 in P. alba×P. berolinensis

A construct containing the open reading frame of OsCYP714D1 (Fig. 1A) was introduced into the genome of the Yinzhong (P. alba×P. berolinensis) hybrid clone by Agrobacterium tumefaciens-mediated transformation. More than 20 independently regenerated lines were obtained and seven transgenic lines (L4, L6, L13, L23, L29, L32, and L33) were identified by PCR analyses. The expected 463bp band was detected in all the checked transgenic lines (Fig. 1B). RT-PCR and GUS staining analyses further confirmed the expression of OsCYP714D1 and GUS in transgenic plants (Fig. 1C, D). Transgenic plants were subsequently transferred to pots and grown in the greenhouse for further experiments.

Constitutive expression of OsCYP714D1 significantly promoted the growth of transgenic plants

Previous studies have shown that when OsCYP714D1 was constitutively expressed in rice (driven by the 35S promoter), transgenic rice showed severe dwarfism and failed to set grain (Zhu et al., 2006). To understand the functions of OsCYP714D1 in poplar development, transgenic poplar plants constitutively expressing OsCYP714D1 were generated. Among the seven independently derived and PCR-confirmed transgenic lines (Fig. 1B), four lines with different OsCYP714D1 expression levels (lines L4, L13, L23, and L32) were chosen for the following studies (Fig. 1C). After 8 weeks growth in the greenhouse, significant growth phenotype differences were observed between WT and transgenic plants (Fig. 2A–D). The growth of three transgenic lines (L4, L13, and L32), which showed significant expression of OsCYP714D1, was faster than that of the WT in terms of plant height (Fig. 3A), leaf number (Fig. 3B), stem diameter (Fig. 3C), stem internode and leaf petiole length (Fig. 3D), and shoot fresh and dry weights (Fig. 3E, F), whereas little difference was found between the WT and transgenic line L23, which showed almost undetectable expression of OsCYP714D1 (Figs 2, 3). To confirm that the OsCYP714D1 gene was inserted into the aspen genome, Southern blot
analyses were performed. Most transgenic plants had 1–2 copies of the transgene (Fig. 2E).

**Constitutive expression of OsCYP714D1 increased xylem cell number, fibre cell wall thickness, and xylem fibre cell length**

A detailed anatomical study of the three transgenic lines L4, L13, and L32 was performed. Compared with the WT control, the transgenic lines showed not only enhanced radial width of the stem and xylem, but also thickened phloem fibre cell walls and an elongated longitudinal length of the outer bark cells (Supplementary Fig S1 at JXB online). The observed differences were further confirmed by measurements by microscopy (Fig. 4). The stem diameter growth was primarily due to the widened xylem since there was no obvious difference between the WT control and transgenic lines in the cambium zone, phloem, and outer bark (Fig. 4A). Moreover, the widened xylem was mainly caused by increased xylem cell number (Fig. 4B). The difference in growth in height in the transgenic plants, compared with the WT control, was primarily caused by the increased lengths of the xylem fibre cells (Fig. 4C) and the collenchyma and parenchyma cells (Fig. 4E). In contrast to the changes in the xylem, the main difference in the phloem of the transgenic plants when compared with the WT control was the thickened phloem fibre cell walls (Fig. 4D).

**GA accumulation was higher in transgenic plants**

GA content was determined in the leaves of actively growing WT and transgenic plants. Transgenic plants showed high levels of the early 13-hydroxylated C\textsubscript{19} GAs (GA\textsubscript{53} and GA\textsubscript{1}) and the non-13-hydroxylated C\textsubscript{19} GAs (GA\textsubscript{12}, GA\textsubscript{9}, and GA\textsubscript{4}). The levels of the biologically active GA\textsubscript{4} in transgenic lines L4, L13, and L32 were pronouncedly higher than in the control plants. The level of GA\textsubscript{1} was relatively low in transgenic lines, but was undetectable in the control plants (Fig. 5).

**Expression of most GA pathway genes was changed in transgenic plants**

The expression of most genes involved in GA biosynthesis and catabolism pathways was examined by quantitative real-time PCR. Four GA biosynthesis genes (three GA20oxs, PtGA20ox1, PtGA20ox3, PtGA20ox4; and one GA3ox, PtGA3ox1), two GA2ox GA catabolism genes (PtGA2ox1 and PtGA2ox2), one GA receptor gene (PtGID1), and two GA suppressor genes (PtRGL1-1 and PtGAI2) involved in GA signalling were analysed (Fig. 6). For the genes involved in GA synthesis, PtGA20ox3 and PtGA3ox1 were down-regulated in transgenic lines L4, L13, and L32 compared with the controls. However, the PtGA20ox4 gene was slightly up-regulated in all four transgenic lines. No distinct change
OsCYP714D1 promotes growth of transgenic trees

Expression of PtCYP714 homologues was down-regulated in transgenic plants

Discussion

As a large group of tetracyclic diterpenes, GAs can be deactivated by conjugation (Schneider and Schliemann, 1994), epoxidation (Zhu et al., 2006), methylation (Varbanova et al., 2007), and 2-oxidation. Map-based cloning has revealed that the EUI gene encodes a previously uncharacterized P450, CYP714D1, which catalyses 16α, 17-epoxidation of non-13-hydroxylated GAs in rice. At the heading stage, the eui mutant exhibited an extremely elongated uppermost
internode and accumulated extremely high levels of bioactive GAs in the uppermost internode. In contrast, transgenic rice overexpressing OsCYP714D1 exhibited a severely dwarfed phenotype (Zhu et al., 2006). Unlike the transgenic rice, transgenic aspen constitutively expressing OsCYP714D1 showed an extremely fast growing phenotype (Figs 2, 3).

Modification of GA biosynthesis has been successfully applied to crop breeding (Sakamoto et al., 2003). GA20ox has been well established as a key enzyme in the biosynthesis of the plant hormone GA, and in the regulation of GA-controlled plant growth (Hedden and Kamiya, 1997). GA20ox-overexpressing plants showed an improved growth rate and biomass, and produced high levels of the 13-hydroxylated C19 GAs (GA20, GA1, and GA3) and the non-13-hydroxylated C19 GAs (GA9, GA4, and GA12) in both internodes and leaves (Eriksson et al., 2000). Overexpression of PtGID1, a GA receptor gene, in transgenic poplars also led to rapid growth, and increased elongation and xylogenesis (Mauriat and Moritz, 2009). Since OsCYP714D1 is involved in the deactivation of GAs (GA4, GA9, and GA12) in rice (Zhu et al., 2006), it was thus speculated that the fast growing phenotypes of OsCYP714D1 transgenic plants could also be attributed to the increased GA production. To this end, the GA contents in both WT and OsCYP714D1 transgenic lines were measured. Similarly, compared with the WT control, OsCYP714D1 transgenic plants accumulated high levels of the early 13-hydroxylated GAs (GA53 and GA4) and the non-13-hydroxylated GAs (GA12, GA9, and GA4) (Fig. 5).
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To clarify the possible reasons for the increased GA accumulation in OsCYP714D1-overexpressing plants, the transcripts of GA pathway genes were investigated (Fig. 6). Complex variations in expression were observed among these genes. Two GA oxidase genes (GA20ox3 and GA3ox1) involved in GA biosynthesis were down-regulated. This could be a consequence of feedback down-regulation by the increased GA accumulation in OsCYP714D1-overexpressing plants. On the other hand, the expression of another GA20ox gene (GA20ox4) was increased in all the transgenic plants, including the negative control line L23, which implies that although GA20ox4 might have a function or regulation mechanism different from GA20ox3, it is not the key factor that increases GA accumulation in transgenic plants. Similarly, different changes in expression were observed with GA2ox1 of OsCYP714D1; L4, L13, and L32, three independent transgenic lines with high expression of OsCYP714D1. Values are means ±SD of three biological replicates of three individual plants from the WT or the same transgenic line. ND, not detected. * indicates significant differences in comparison with the WT at P < 0.05 (Student’s t-test).

Fig. 4. Effects of OsCYP714D1 expression on stem radial growth, xylem cell number, fibre cell wall thickness, and outer bark cell and xylem fibre cell length of 2-month-old wild-type (WT) and OsCYP714D1 transgenic plants (lines L4 and L32). (A–E) Average values with standard deviations for radial width (A), xylem cell number (B), xylem fibre length (C), cell wall thickness of xylem fibres (Xf) and phloem fibres (Pf) (D), and cell length of outer bark (E). Values shown are means ±SD of at least 10 tissues (for radial width and xylem cell number) or 30 cells (for cell wall thickness, cell length of outer bark) or 300 cells (for xylem fibre length) of WT and transgenic plants (L4 and L32). T, total value of xylem, cambium, phloem, and outer bark; OB, outer bark; P, phloem; Ca, cambium; X, xylem; Xf, xylem fibre; Pf, phloem fibre; Ep, epidermis; Co, collenchyma; Pa, parenchyma. * indicates significant differences in comparison with the WT at P < 0.001 (Student’s t-test).

Fig. 5. Endogenous GA levels in the leaves of 2-month-old wild-type and OsCYP714D1 transgenic plants. WT, wild type; L23, a negative control transgenic line with very low expression of OsCYP714D1; L4, L13, and L32, three independent transgenic lines with high expression of OsCYP714D1. Values are means ±SD of three biological replicates of three individual plants from the WT or the same transgenic line. ND, not detected. * indicates significant differences in comparison with the WT at P < 0.05 (Student’s t-test).
and GA2ox2, two GA2ox genes involved in GA catabolism (Busov et al., 2003). The slight down-regulation of GA2ox2 in OsCYP714D1-overexpressing lines might be caused by the feedback of OsCYP714D1. No significant difference in GA2ox1 expression was observed between the WT and the transgenic plants, except for L32, in which the expression of GA2ox1 was significantly enhanced. This result might be attributed to the different insertion sites and copy numbers of the exogenous gene among the different transgenic lines. Similar results were also observed in the other analysed genes such as GID1 and GAI2. The soluble GA receptor (GID1) and DELLA proteins are the key mediators of GA response pathways. DELLA proteins act as negative regulators and are degraded in response to GA treatment (Ueguchi-Tanaka et al., 2007). The expression of PtGID1 and two putative DELLA protein genes, PtRGL1-1 and PtGAI2, was also detected. The increased GA accumulation up-regulated the expression of PtGID1 in transgenic lines L4 and L32, and, at the same time, down-regulated the expression of PtRGL1-1 in L4 and L13, as well as of PtGAI2 in L13.

Although the expression levels of most GA pathway genes changed differently in the WT and transgenic plants, it seems that the differences were more likely to be the consequence but not the cause of the increased GA production in the OsCYP714D1-overexpressing plants. In P. deltoides hybrids and other Populus species, different GA members of both the early 13-hydroxylation and non-early 3- or 13-hydroxylation pathways of GA biosynthesis have been detected (Bate et al., 1988; Rood et al., 1988; Zanewich and Rood, 1994; Eriksson et al., 2000; Pearce et al., 2002). Therefore, different GA metabolites and different predominant biosynthesis pathways may exist in different Populus species. The unexpected fast growing phenotype and high levels of GA accumulation could be caused by the different function of OsCYP714D1 in aspen. OsCYP714D1 may use different substrate(s) in aspen which lead to a higher GA content. Indeed, transgenic plants overexpressing the Arabidopsis Eui gene (AtCYP714A1 and AtCYP714A2) also showed different phenotypes from plants overexpressing OsCYP714D1 when treated with exogenous GAs (Zhang et al., 2011). In addition, the CYP714 proteins in aspen share very high homology with OsCYP714D1 and AtCYP714s (Supplementary Fig. S2 at JXB online). Therefore, one of the other possibilities is that constitutive expression of OsCYP714D1 suppressed the expression of PtCYP714 genes in the transgenic aspen. The down-regulated expression of most PtCYP714 genes in transgenic plants seems to support this hypothesis (Fig. 7). The down-regulated expression of putative orthologous Populus CYP714 genes
may also be a consequence of GA production. Further work on the functional characterization of these CYP714 family members in aspen will help to elucidate whether GA 16α, 17-epoxidation is a common GA deactivation reaction in the GA metabolism pathway in woody plant species.

It has been well documented that GAs are required for xylem fibre cell differentiation (Wareing, 1958; Digby and Wareing, 1966). They profoundly affect the development of secondary xylem fibres as well as both longitudinal and radial growth in hardwood species (Little and Pharis, 1995; Ridoutt et al., 1996) and conifers (Wang et al., 1995). In the present experiments, overexpression of OsCYP714D1 in transgenic poplars increased GA contents, and, as a result, increased xylem cell number, and xylem fibre and cortex cell length in the stems. In addition, GA and IAA combination, not GA alone, could affect primary phloem fibre differentiation, including the thickness of phloem fibre cells in Coleus blumei (Aloni, 1979). It has also been suggested that a cross-talk between GA and auxin (IAA) exists during the growth and development of poplars (Björklund et al., 2007; Gou et al., 2010; Mauriat et al., 2011). Thus, to clarify the reason for the phloem fibre cell wall thickness in transgenic poplars (Supplementary Fig. S1G–I at JXB online), the endogenous IAA content was measured (Supplementary Fig. S3). As expected, the IAA content increased distinctly in the transgenic plants compared with the WT control. This might be attributed to the increased auxin polar transportation in transgenic plants (Björklund et al., 2007). Taken together, constitutive expression of the OsCYP714D1 gene increased GA and IAA production, and consequently promoted the growth of transgenic plants. The substantially increased growth and biomass reported here provide a promising strategy for increasing wood production in plants.

**Supplementary data**

Supplementary data are available at JXB online.

**Figure S1.** Cellular morphology of the stems of 2-month-old wild-type and OsCYP714D1 transgenic plants (lines L4 and L32).

**Figure S2.** The encoded sequences of PtCYP714A3, PtCYP714E2, PtCYP714E4, PtCYP714E5, and PtCYP714F1 from *Populus trichocarpa* genotype Nisqually-1 were aligned with OsCYP714D1 from rice, and AtCYP714A1 (At5g24910) and AtCYP714A2 (At5g24900) from *Arabidopsis*.

**Figure S3.** Endogenous IAA levels in the leaves of 2-month-old wild-type and OsCYP714D1 transgenic plants.

**Table S1.** Gene-specific primers used in this study.
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