Plant Soil (2018) 425:309–319
https://doi.org/10.1007/s11104-018-3583-z

REGULAR ARTICLE

Arbuscular mycorrhizal fungi increase the proportion of cellulose and hemicellulose in the root stele of vetiver grass

Xun Wen Chen · Yuan Kang · Pui San So · Charles Wang Wai Ng · Ming Hung Wong

Received: 17 December 2017 / Accepted: 24 January 2018 / Published online: 13 February 2018
© Springer International Publishing AG, part of Springer Nature 2018

Abstract
Aims *Arabidopsis thaliana* is the model plant that is mainly used in studying cellulose and hemicellulose (CH) biosynthesis. Unfortunately, *A. thaliana* does not associate with mycorrhiza and as a result there are only rare reports on the role of arbuscular mycorrhiza (AM) fungi on CH biosynthesis. This study aims to investigate the effects of AM fungi on changing the CH content in mycorrhizal plant.
Methods Three AM fungi, *Glomus aggregatum*, *Rhizophagus intraradices* and *Funneliformis mosseae*, were inoculated to vetiver grass (*Chrysopogon zizanioides*) and grown for 12 months. Roots were harvested, and the proportions of CH, lignin, lipids and hydrosoluble content were analysed. The corresponding root tensile strength (positively correlated with the proportion of CH) was measured to counter check the CH content.
Results Plants inoculated with AM showed a higher proportion of CH ($P < 0.05$) compared with uninoculated ones. This increase was coupled to a 40–60% enhancement in tensile strength. Potential mechanisms for this phenomenon are discussed.
Conclusions This is the first study showing that the proportion of CH and tensile strength of plant root could be significantly affected by AM symbiosis. It is thus desirable that future research on CH biosynthesis uses mycorrhizal-associating plants, such as medic (*Medicago truncatula*) and rice (*Oryza sativa*).

Responsible Editor: Tatsuhiro Ezawa

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11104-018-3583-z) contains supplementary material, which is available to authorized users.

X. W. Chen · P. S. So · C. W. W. Ng (✉) ·
M. H. Wong (✉)
Department of Civil and Environmental Engineering, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, SAR, China
e-mail: cecwwng@ust.hk
e-mail: minghwong@eduhk.hk

Y. Kang
School of Chemistry & Environment, Higher Education Mega Center, South China Normal University, Guangzhou 510006, China

M. H. Wong
Consortium on Health, Environment, Education and Research (CHEER), and Department of Science and Environmental Studies, The Education University of Hong Kong, Tai Po, Hong Kong, SAR, China

M. H. Wong
School of Environment, Jinan University, Guangzhou, China

Keywords Cellulose synthase · Glycosyltransferase · Arbuscular mycorrhiza · Root · Tensile strength · *Chrysopogon zizanioides*

Introduction

Understanding the mechanism of cellulose biosynthesis is one of the challenges in plant biology. Cellulose, a crystalline glucan, is the most abundant biopolymer in the world, and it presents mostly in the plant cell wall.

This biomass serves as a global carbon sink, although the carbon can be released due to cellulose-induced microbial priming effects (Heimann and Reichstein 2008). Cellulose is also a sustainable resource for biofuel (Weng et al. 2008) and fibre industry (Reddy and Yang 2005; Kalia et al. 2011). Cellulose productivity and its mechanical properties are essential for investigating plant morphogenesis (Bidhendi and Geitmann 2016; Xiao et al. 2016) and applications in fibre industry. Although cellulose is considered as a resource for biofuel manufacture, lignin is a limiting component which should be removed beforehand (Weng et al. 2008; Li et al. 2008; Weng and Chapple 2010). Therefore, the proportion of cellulose, hemicellulose and lignin in a given biomass is a good proxy of the costs of cellulose extraction during biofuel production (Boerjan et al. 2003). In addition, understanding the mechanical properties of plants (related to the proportion of cellulose and hemicellulose, see Genet et al. 2005) can enable engineers to apply plant materials (e.g., plant roots) for stabilizing slopes (Pollen and Simon 2005; Wu 2013) and in bamboo for construction applications (Xiao et al. 2010; Youssefian and Rahbar 2015), which are more eco-friendly compared to traditional methods. Understanding the mechanism of plant cell formation and its properties is of fundamental and general interest to plant biologists, environmental scientists and engineers.

Previous studies have investigated the mechanism of cellulose biosynthesis using the model plant *Arabidopsis thaliana* (Arioli et al. 1998; Watanabe et al. 2015), a cellulose producing bacterium *Acetobacter xylinum* (Ross et al. 1991), or by comparing the cellulose synthase (CesA, catalyses the reaction that synthesizes glucose to glucan chain) gene expressions in *A. thaliana* and maize (Holland et al. 2000). Such studies were confined to laboratory experiments and did not consider the role of biotic and abiotic factors, which are ubiquitous in ecosystems and agricultural fields. Among all the biotic factors, the symbiosis between plants and arbuscular mycorrhizal (AM) fungi is considered to play an important role in ecology (van der Heijden et al. 1998), global carbon distribution (Treseder and Allen 2000; Cheng et al. 2012; Solaiman 2014), and stress tolerance of plants (Smith and Read 2008). Unfortunately, the model plant *A. thaliana* used for studying cellulose biosynthesis is considered as a non-mycorrhizal plant (Veiga et al. 2013). Studying the plant cell wall formation without considering the role of AM fungi only shows part of the picture.

Increases in plant biomass upon AM symbiosis represent a common outcome of AM experiments (Smith and Read 2008; Hoeksema et al. 2010; Bonfante and Genre 2010). However, the changes of the cellulose proportions (the percentage of the cellulose content in a certain amount of biomass) upon AM symbiosis, related to the deposition or orientation mechanism during cellulose synthesis, have not been reported. Modification of the plant cell wall has also been found upon AM symbiosis (Rich et al. 2014). Hydroxyproline-rich glycoprotein (HRGP) and cellulose have been localized in the cell wall and interface area created by invagination of the host membrane around the developing fungus. In contrast, in non-mycorrhizal roots, HRGP and cellulose appear only in the peripheral region of the cell wall, in close contact with the plasma membrane (Bonfante et al. 1990; Balestrini et al. 1994). The cell wall material is laid down between the host plasma membrane and the fungal cell surface (Balestrini and Bonfante 2014). The thickness of the cortical cell wall has been found to significantly increase due to AM fungal colonization (Balestrini et al. 2005). However, these studies were more focused on cell wall modification in the AM fungi-containing tissue (i.e. root cortex), and did not pay attention to other tissues, such as root stele.

Furthermore, expression of the genes involved in cell wall metabolism has been found to be enhanced in mycorrhizal roots (Guether et al. 2009; Fiorilli et al. 2009). Part of the photosynthate (glucose) is used to synthesize cellulose and hemicellulose (CH) with glycosyltransferases (GTs) in plants (Taylor 2008; Moran et al. 2011; Pauly et al. 2013). GTs are a ubiquitous group of enzymes that catalyse the transfer of a sugar moiety from an activated sugar donor onto saccharide or non-saccharide acceptors (Coutinho et al. 2003). On the other hand, 4–20% of the photosynthate (glucose) in plants would be acquired by AM fungi and transformed to lipids and trehalose (Smith and Read 2008). Thus, the allocation of glucose (building blocks of CH) may be influenced by AM symbiosis. Additionally, inoculation of AM fungi could increase the expression of GTs (Detering et al. 2005; Fiorilli et al. 2009), the genes related to cell wall metabolism (Guether et al. 2009) and CesA (Siciliano et al. 2007; Vangelisti et al. 2018). It has been also reported that expression of *MtCel1*, responsible for the assembly of cellulose/hemicellulose matrix, associated specifically with cells containing arbuscules (Liu et al. 2003).

Such modifications could potentially change the amount and proportion of cellulose, hemicellulose, lipids and the hydrosoluble contents (e.g., inorganic salts, see Rowell (1984)). Taken together, the influence of AM fungi on both CH synthesis and proportion at systemic level are underexplored. Therefore, the objectives of the present study are to investigate whether the inoculation of AM fungi would increase the proportion of CH content in roots, and to what extent would the root tensile strength be enhanced.

Materials and methods

Plant and AM fungi

Vetiver grass (*Chrysopogon zizanioides*) was selected since it possesses a significant amount of root biomass, and forms symbiosis with AM fungi (Wong et al. 2007). The inocula of three AM fungal species, *Rhizophagus intraradices* (Ri, BGC BJ09), *Funneliformis mosseae* (Fm, BGC HK01) and *Glomus aggregatum* (Ga, BGC HK02D), obtained from the Beijing Academy of Agriculture and Forestry Sciences, were used as the symbionts of vetiver grass. The granular inoculum is a mix of root pieces, mycelium pieces and spores.

Experimental setup

Completely decomposed granite (CDG) soil was used as the growth substrate for the grass. CDG is soil weathered from granite. It is commonly found in some tropical and sub-tropical regions (e.g., granite occupies >85% of land in Hong Kong) (GEO 2000), and is used for general and slope landscaping (GEO 2011). The gravel, sand, silt and clay contents of CDG were 19, 42, 27 and 12%, respectively. The pH of CDG was 6.0. The concentration of extractable N, P, K and total C of CDG were 0.80 mg kg^{-1}, 0.81 mg kg^{-1}, 15.2 mg kg^{-1} and 1.2%, respectively. Soil properties were analysed according to the methods described by Klute et al. (1994) and Sparks et al. (1996).

Sterilized (autoclaved at 121 °C for 2 h) CDG was compacted in each PVC pot (diameter × height = 200 mm × 400 mm). For compaction, sterilized soil was mixed with deionized water to achieve the optimal water content (13%) (Ng et al. 2013). The maximum dry density of CDG is around 1870 kg m^{-3} (Ng et al. 2013). The soil in each pot was compacted in 13 layers (28 mm each) (ASTM 2007). The final soil dry density was approximately 1780 kg m^{-3} (95% of compaction). The objective for achieving a high compaction rate was to investigate whether AM fungi can enhance the tensile strength of roots grown on slope (95% of compaction is required due to safety concerns, see GEO 2011), and further enhance slope stability (Wu et al. 1979; Chen et al. 2016).

Twenty grams of inoculum of each AM fungus was added uniformly as a layer in each pot at a 20 mm depth from the soil surface upon planting. Sterilized inoculum (autoclaved at 121 °C for 2 h) was also added to a separate pot as a control (non-mycorrhizal, NM). Three slips of vetiver grass of approximately uniform size (~20 cm tall) were transplanted to each pot with the roots directly contacting the inoculum. The setup was a completely randomized experimental design with five replicates and the type of mycorrhizal inoculation as the only experimental factor. There were 20 pots and 60 individuals of vetiver grass in total. The plants were allowed to grow outdoors for 12 months, subjected to the changes of natural sunlight, humidity (70–90%), and temperature (15–29 °C). The position of each pot was changed randomly each week. The same volume of half strength Hoagland nutrient solution (Hoagland and Arnon 1950), with limited phosphorus (1/5 of the concentration), was added to each pot every two weeks.

Harvesting and root tensile strength measurement

During harvesting, the soil attached to the root was gently removed using water to avoid root damage. The roots and shoots from each pot were separated, dried with paper tissue, and weighed for wet biomass. All the shoots were oven-dried at 60 °C for 72 h and weighed to determine the dry mass (Cornelissen et al. 2003). The roots were soaked in 15% alcohol for storage prior to the tensile strength measurements, which were completed within two months before root decomposition (Genet et al. 2005; Baets et al. 2008).

Root samples of length between 6 and 7 cm were randomly sampled for the tensile strength measurement using a universal testing machine (EZ50, Lloyd Instruments), modified with a 50 N load cell with accuracy ±0.5% (XLC-0050-A1) and a vice grip set (01/4215). To avoid slippage of the roots out of the clamps, thin slices of rubber (1.5 cm × 1.5 cm) were fixed between the jaws and the root. The distance between the two jaws was set at 3 cm at the initial stage. 1.5–2.0 cm of the root

length within each jaw was used for clamping purposes. During testing, the displacement of the crosshead of EZ50 was controlled at a constant velocity of 4 mm min^{-1} (Tosi 2007). Breaking the root at the point contacting the clamp was not considered as a valid measurement (Genet et al. 2005; Tosi 2007). In total, 170, 242, 257 and 225 roots (894 in total) were successfully measured for NM, Ga, Ri and Fm treatments, respectively. Details of the sample sizes are shown in Table S1. The force on breaking and the breaking point diameter of the root (and stele) were recorded to calculate the tensile strength:

$$Tensile\ strength = \frac{Maximum\ force\ on\ breaking}{Cross\ sectional\ area\ at\ breaking\ point}$$

AM fungal colonization rate, root stele CH, lignin, lipids and hydrosoluble content measurements

Subsamples of the root were collected and stained, and the colonization rate of the AM fungi was assessed using the slide length method (Giovannetti and Mosse 1980). For each pot, 60 pieces of root (length: 1 cm each) were randomly selected and observed under a microscope (Ni-U, Nikon Corporation, Japan; software: MBF Stereo Investigator).

Since the stele of the root contributes to the ultimate tensile strength, the CH proportion in the steles was determined using the method described in previous studies (Leavitt and Danzer 1993; Genet et al. 2005). Briefly, the cortex of the roots was carefully removed with a scalpel. The steles collected were oven-dried at 60 °C for 72 h (Cornelissen et al. 2003) and ground into fine power using a ball mill (8000 M Mixer/Mill, SPEX SamplePrep, UK). The powder was poured into a Teflon sachet (pore size 1.2 μm) labelled with the sample ID and weighed using a balance with a precision of 0.0001 mg. The sachets without sample were treated with toluene 99%-ethanol 96% (2–1; *v*/v) for 24 h to remove potential contaminants beforehand.

To remove the lipids, the samples were treated using a Soxhlet extractor with toluene 99%-ethanol 96% (2–1; v/v) for 24 h, and followed by ethanol for another 24 h. The hydrosoluble content was removed by dissolving it in distilled water at 100 °C for 6 h. Since lignin also contributes to the tensile strength (Zhang et al. 2013, 2014), all samples were dried and weighed to measure the total mass of CH and lignin after removing the above-mentioned lipids and hydrosoluble content. To eliminate lignin, the samples were further immersed in a solution containing 700 ml of distilled water, 7.0 g of sodium chlorite and 1.0 ml of acetic acid, and heated to 70 °C for 12 h. This process was repeated three times. Finally, the sachets with the samples were dried and weighed. The proportions of CH, lignin, lipids and hydrosoluble content were calculated from the relative differences before and after the extraction.

Statistical analyses

All statistical analyses were performed using SPSS v22.0 software. The normality of data was checked by using the Shapiro-Wilk test. Data was log-transformed to meet the normality assumption when necessary. Levene's test was used to assess the homogeneity of the data. To test whether the differences in the proportions of various root components and the tensile strength were significant between treatments (factor: different AM fungal species.), one-way analysis of variance (ANOVA) followed by the Duncan's multiple range post-hoc test were conducted at a significance level of 5%.

Results

Plant biomass and AM fungal colonization rate

Compared with the control, the roots wet masses were significantly increased ($P < 0.05$) in all mycorrhizal treatments, while the shoot wet mass was only increased in Ga treatment (Fig. 1a). Ri and Fm had no significant effect on the root and shoot dry mass, whereas Ga significantly increased ($P < 0.05$) both the root and shoot dry mass (Figs. 1b and c). The AM colonization rates of the plants inoculated with AM fungi were <5% on average. AM structures in the cortex of the roots, including arbuscules, vesicles and hyphae, were observed (Fig. 2).

AM fungi enhanced the tensile strength of root via increasing the percentage of CH

During tensile strength measurement, it was observed that the cortex broke at the beginning of the test. Subsequently, the stele withstood the ultimate breaking force, but not the cortex, indicating that the cortex

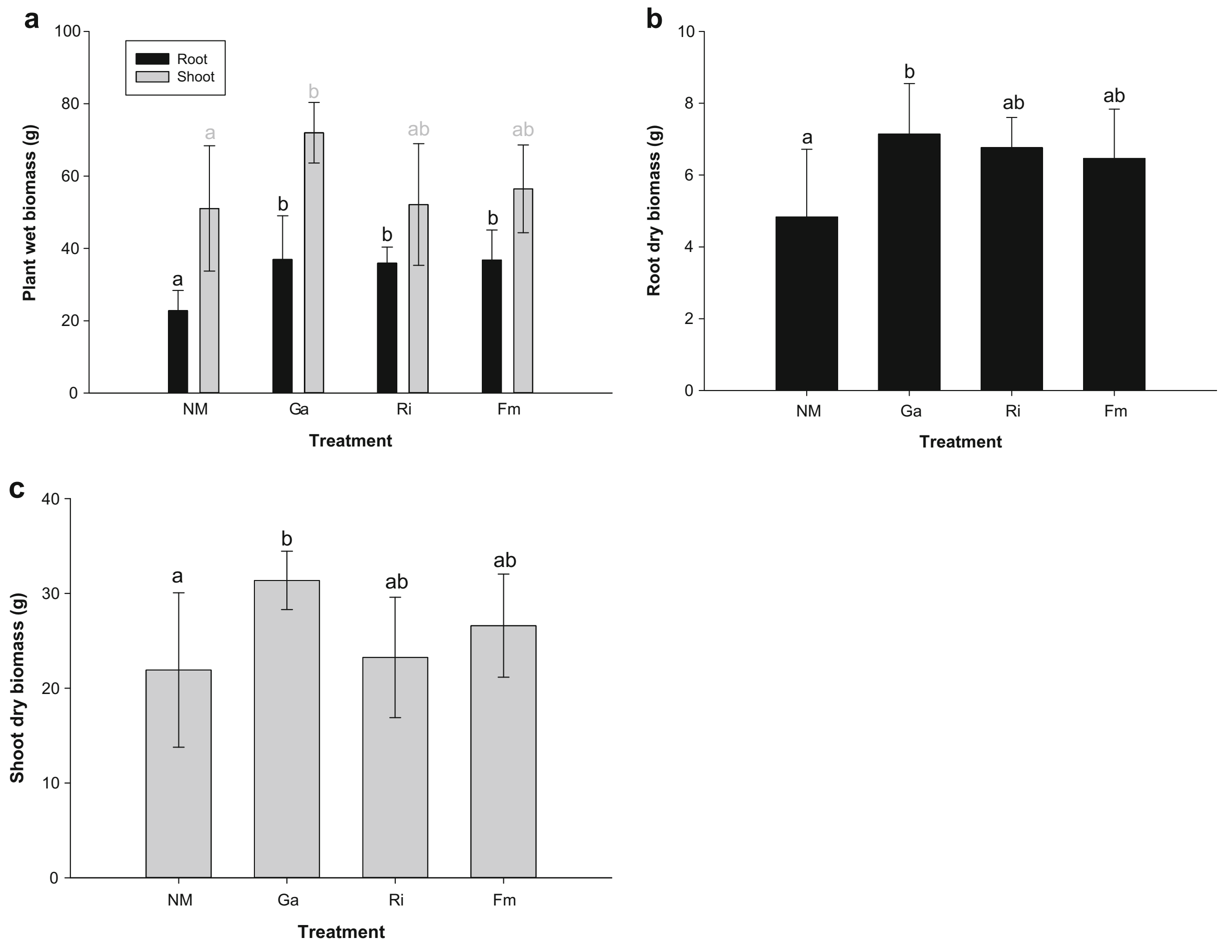

Fig. 1 Data on plant wet (**a**) and dry (**b** and **c**) biomass in four different treatments. Different letters above bars indicate significant differences among treatments. NM, Ga, Ri and Fm represent the grasses inoculated with sterilized inoculum (control), *G. aggregatum*, *R. intraradices* and *F. mosseae*, respectively. Duncan's multiple range test at a probability level of 5% was used for post-hoc comparison to separate the differences. Data are mean ± S.D. ($n = 5$)

(harbouring AM fungi) did not contribute to the tensile strength. The relationship between the diameters of root and stele is shown in Fig. S1. The tensile strength in the stele diameter ranges of 0.1–0.2 and 0.2–0.3 mm (Fig. 3) was significantly higher ($P < 0.05$) in all AM treatments. In the range of 0.3–0.4 mm, only the Ri treatment increased the tensile strength. For the ranges of 0.4–0.5 and 0.5–1.0 mm, both Ri and Fm treatments enhanced the tensile strength, but not the Ga treatment. These indicate that the AM treatments could generally enhance the stele tensile strength, especially for fine steles (0.1–0.3 mm). The AM fungal structures were only found in the fine roots (stele diameter 0.1–0.3 mm) of the present study.

Tensile strength has been reported to positively correlate with the percentage of CH in the material (Genet et al. 2005). In order to confirm the finding that AM fungal treatments enhance tensile strength, the proportions of CH in the corresponding steles were measured. Higher percentages of CH ($P < 0.05$) were observed in the treatments with AM fungal inoculation (green letters above the error bars, Fig. 4). The proportion of lipids and hydrosoluble content was decreased (red letters), and this was compensated only by CH, but not lignin (black letters). Fig. S2 shows the relation between root tensile strength and root stele diameter. For all roots, smaller stele diameters led to higher levels of tensile strength, which can be represented by power equations (Fig. S2).

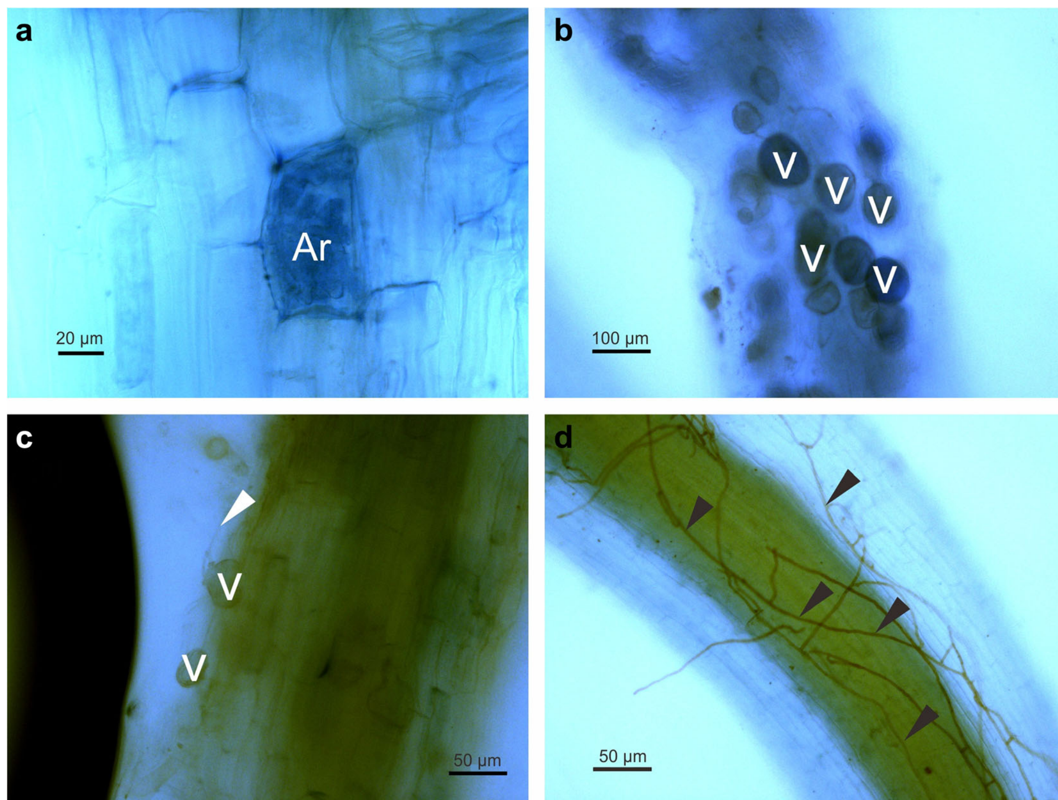

Fig. 2 AM fungal structures observed in roots inoculated with AM fungi Ga (**a**), Ri (**b**) and Fm (**c** and **d**). Ar, arbuscule; v, vesicle; arrow, hyphae. Ga, Ri and Fm refer to *G. aggregatum*, *R. intraradices* and *F. mosseae*, respectively

Discussion

The relatively low levels of colonization (~ 5%), compared with those reported by Wong et al. (2007), which ranged between 30 and 65%, did not hinder the AM fungi from contributing to increased root wet mass (Fig. 1a). The low colonization rate was probably caused by the high soil density (dry density 1780 kg m^{-3}) imposing a

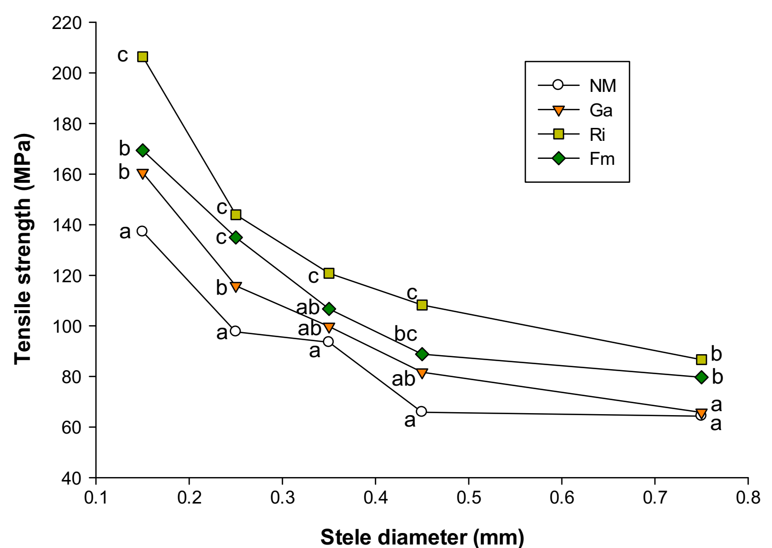

Fig. 3 Comparison of root tensile strength derived from different mycorrhizal treatments and different classes of root stele diameter. Different letters indicate significant differences among treatments for each class of stele diameter. Duncan's multiple range test at a probability level of 5% was used for post-hoc comparison to separate differences. Data points are average values. Sample size for each class and treatment is shown in Table S1. NM, Ga, Ri and Fm refer to the grasses inoculated with sterilized inoculum (control), *G. aggregatum*, *R. intraradices* and *F. mosseae*, respectively

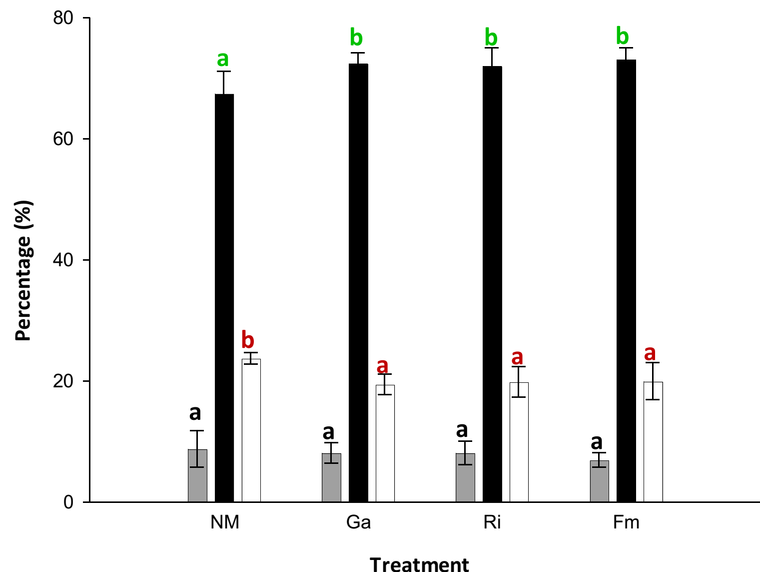

Fig. 4 Comparison of the percentages of lignin (grey bar), cellulose + hemicellulose (black bar), and lipid + hydrosoluble contents (open bar) in the steles of roots derived from different mycorrhizal inoculations. Different letters above bars indicate significant differences among treatments. Duncan's multiple range test at a probability level of 5% was used for post-hoc comparison to separate differences. Data are mean ± S.D. (n = 5). NM, Ga, Ri and Fm represent the grasses inoculated with sterilized inoculum (control), *G. aggregatum*, *R. intraradices* and *F. mosseae*, respectively

negative impact on the colonization, which has been reported in Trifolium (Nadian et al. 1996). The soil bulk density of 1750 kg m^{-3} decreased the colonization rates of several AM fungal species in Trifolium from 25 to 70% to 10% (Nadian et al. 1998). In our case, the soil density was even higher than 1750 kg m^{-3}, and may further reduce the colonization to our present result of about 5%. In addition, it has been shown that the colonization rate displays seasonal patterns which are potentially related to the climate and the life cycle of the species (Mayr and Godoy 1990), such that the AM colonization rate could fluctuate dramatically in different seasons (Bencherif et al. 2016). Thus, colonization rate may not be a determining factor for evaluating the contribution of AM fungi.

Increases in plant mass have been commonly observed after AM fungal treatment (Wong et al. 2007; Smith and Read 2008). The root mass can be promoted by inoculation of AM fungi (Smith and Read 2008), thus the total amount of CH can be enhanced, but the proportion of each content can be unchanged. However, our results show significant increases in CH proportions, not only the actual amount (i.e. mass). The percentage of CH is positively correlated to the tensile strength (Genet et al. 2005), calculated by using the ultimate breaking force divided by the cross-sectional area of the material. Tensile strength is a normalized parameter representing the force in one unit of area. It should not be affected by the size of the root/plant. This validates the comparison between plants with different sizes. The density and bonding of cellulose microfibrils (composed of glucan chains) and xylan chains (hemicellulose) in each unit of the cross-sectional area seem to be essential parameters affecting tensile strength.

We found that the lower lipid and hydrosoluble content in our AM plants was partially compensated by the CH, but not lignin (Fig. 4). The phenomenon in which cellulose and lignin deposition regulated in a compensatory fashion (more cellulose led to less lignin, vice versa) in transgenic aspen (*Populus tremuloides*, dicot) (Hu et al. 1999) was not observed in the present study. However, our results are in line with the qualitative observation on a monocot (leek, *Allium porrum*), that a compensatory fashion between cellulose and pectic components was observed in root cell wall due to mycorrhizal symbiosis (Bonfante et al. 1990).

Increase of cell wall thickness during AM colonization could be caused by the loosening required for the fungal intracellular colonization and/or synthesis of new structural polymers (Balestrini and Bonfante 2005). The strength of the root is derived from the organization of cellulose microfibrils which are related to the degree of polymerization and microfibril crystallinity (Saxena and Brown 2005; Joshi and Mansfield 2007). It is not clear whether the loosening can affect the organization of cellulose microfibrils which subsequently affect tensile strength. In the present study, the tensile strength was enhanced more significantly in the fine roots (stele diameter 0.1–0.3 mm) in Ga and Fm treatments. This may

be due to the fact that AM fungi can easily colonize fine roots, rather than coarse roots (Wu et al. 2016), promoting GT and CesA expression (Guether et al. 2009; Fiorilli et al. 2009). This may enhance the cellulose biosynthesis, and thus increase the tensile strength. It should be noted that the Ri treatment significantly increased the tensile strength of both fine and coarse roots.

We postulate that, upon AM fungal colonization, more cellulose synthase complexes (CSCs) (synthesizing cellulose microfibrils) are assembled in the Golgi apparatus (Wightman and Turner 2010), leading to a greater number (or density) of complexes transferred/activated to/in the plasma membrane. This process involves (1) the enhancement of CesA expression (e.g., CesA was found exclusively in arbuscule-containing cells in *Lotus* (Guether et al. 2009)), (2) the efficiency of the Golgi in complex production, (3) the trafficking activity of the complexes from Golgi to the plasma membrane (Bashline et al. 2014), (4) activation of the complexes, and (5) more glucose being photosynthesized for glucan chain production. Such a process may increase the number of rosette subunits (formed by six CesA) and thus the number of rosettes (formed by six rosette subunits) located in the plasma membrane. The CesA assembles more glucose into a glucan chain, and the rosettes are responsible for synthesizing these glucan chains into cellulose microfibrils via hydrogen bonding (Taylor 2008). Sucrose synthase (SuSy) produces and transports uridine diphosphate (UDP) glucose to the plasma membrane (Amor et al. 1995; Coleman et al. 2009; Zheng et al. 2011). UDP-glucose located in the plasma membrane is used by CesA as a substrate to produce glucan chains. SuSy belongs to the GT family. It has been evidently shown that GT expression was 7.4-fold up-regulated upon colonization of an AM fungus (*F. mosseae*) in tomato roots (Fiorilli et al. 2009). It also has been found that genes encoding GTs were changed due to AM fungal colonization in *Medicago* (Detering et al. 2005). Fourteen genes involved in cell wall metabolism were found to be up-regulated upon mycorrhizal symbiosis in *Lotus* (Guether et al. 2009). The GTs facing the Golgi lumen (i.e. Golgi-localized STELLO proteins) were found to regulate the assembly and trafficking of CSCs in *A. thaliana* (Zhang et al. 2016).

For commelinid monocots (e.g. vetiver), the xylan (specifically glucuronoarabinoxylan, GAX) is the major hemicellulose in the primary cell wall (20–40% of xylan by weight) and the secondary cell wall (40–50% of xylan) (Scheller and Ulvskov 2010). Unlike cellulose biosynthesis, hemicellulose is synthesized through different routes. Mutants with abnormal xylan biosynthesis have a collapsed xylem phenotype which impairs the resistance to the negative pressure generated by transpirational pull (Pauly et al. 2013). This indicates that xylan, in addition to glucan in the cellulose, contributes to the tensile strength as well. Several GTs, including IRX9 (GT family 43), IRX14 (GT family 43), and IRX10 (GT family 47), have been identified and are thought to be involved in xylan backbone elongation (Scheller and Ulvskov 2010; Pauly et al. 2013). The GAX synthase complex in wheat is formed cooperatively by GTs from families 43, 47 and 75 (Zeng et al. 2010). It is clear that the expression of GT could be up-regulated by AM fungi (Fiorilli et al. 2009), and GTs play essential roles in both cellulose and hemicellulose biosynthesis.

Plant cell wall structure can be changed upon AM symbiosis (Rich et al. 2014). Upon AM fungal colonization in the cortex, the HRGP and cellulose were localized in both the wall and the interface area created by invagination of host membrane around the developing fungus. On the contrary, HRGP and cellulose were only found to be present in the peripheral region of the cell wall in uninfected roots (Bonfante et al. 1990; Balestrini et al. 1994). Cell wall material is laid down between the host plasma membrane and fungal cell surface (Balestrini and Bonfante 2014). These studies, however, only investigated the cell wall in the cortex, where harbours AM fungi. It has been stated that AM fungi never penetrate the central cylinder (i.e. stele) (Bonfante et al. 1990). Information on the stele cell wall, in which the CH proportion and tensile strength were increased in the present study, is lacking. The increase of CH proportion seems to be a systemic, rather than a local phenomenon, since the CH proportion was increased in the stele which does not harbour AM fungi.

An alternative mode of CH biosynthesis upon AM symbiosis has not yet been proposed. Such a mode can be induced by the re-regulation of GT expression due to AM symbiosis, which can be similar to the re-regulation of Pi transporter expression in mycorrhizal plants (Paszkowski et al. 2002; Karandashov and Bucher 2005; Javot et al. 2007). Studies using bacteria and plant mutants (mostly *A. thaliana*) to investigate CH biosynthesis did not consider the involvement of AM fungi. *A. thaliana* is considered as a non-mycorrhizal plant (Veiga et al. 2013). Instead, it would be worthwhile to

study the mycorrhizal mutants (rice or medic), such as knockouts of the genes RSW1 (corresponding mutant *rsw1* causes radial swelling phenotype) (Arioli et al. 1998), CesA1–9 (Holland et al. 2000) and IRX9, 10 and 14 (Scheller and Ulvskov 2010), to find out whether the mycorrhizal mutants can produce strengthened cell walls compared to non-mycorrhizal mutants. The role of the AM fungi in CH biosynthesis requires more in-depth exploration on the mechanism at the molecular level.

Conclusions

The mechanism of CH biosynthesis in higher plants has long been studied, mostly using *A. thaliana*. However, *A. thaliana* is considered non-mycorrhizal. The role of AM fungi in plant cell wall formation has been neglected. The three AM fungal species used in the present study had significant effects ($P < 0.05$) on increasing the root wet mass and the proportion of CH content in the root stele of vetiver grass. This was confirmed by the enhancement of the root tensile strength. The most effective AM species in increasing root tensile strength is *R. intraradices* (Ri), followed by *F. mosseae* (Fm) and *G. aggregatum* (Ga). The AM fungi tended to increase the tensile strength of roots with smaller stele diameters (0.1–0.3 mm), and to play an important role in plant cell wall formation. AM fungi have the potential to improve CH biosynthesis. Future study should focus on investigating the mechanism of AM fungi in affecting the biosynthesis at the systemic level (e.g., verifying the effects on the aerial part of plant, which is of interest for biofuel production) using mycorrhizal model plants.

Acknowledgements The financial support from the Collaborative Research Fund of the Research Grants Council, Hong Kong SAR (HKUST6/CRF/12R) is gratefully acknowledged.

References

Amor Y, Haigler CH, Johnson S et al (1995) A membrane-associated form of sucrose synthase and its potential role in synthesis of cellulose and callose in plants. Proc Natl Acad Sci U S A 92:9353–9357

Arioli T, Peng L, Betzner AS et al (1998) Molecular analysis of cellulose biosynthesis in *Arabidopsis*. Science 279:717–720

ASTM (2007) Test Methods for Laboratory Compaction Characteristics of Soil Using Standard Effort (12 400 ft-lbf/ft^3 (600 kN-m/m^3)). ASTM standard D698. American Society for Testing and Materials, West Conshohocken

Baets SD, Poesen J, Reubens B et al (2008) Root tensile strength and root distribution of typical Mediterranean plant species and their contribution to soil shear strength. Plant Soil 305: 207–226

Balestrini R, Bonfante P (2005) The interface compartment in arbuscular mycorrhizae: A special type of plant cell wall? Plant Biosyst Int J Deal Asp Plant Biol 139:8–15

Balestrini R, Bonfante P (2014) Cell wall remodeling in mycorrhizal symbiosis: A way towards biotrophism. Front Plant Sci 5:1–10

Balestrini R, Romera C, Puigdomenech P, Bonfante P (1994) Location of a cell-wall hydroxyproline-rich glycoprotein, cellulose and β-1,3-glucans in apical and differentiated regions of maize mycorrhizal roots. Planta 195:201–209

Balestrini R, Cosgrove DJ, Bonfante P (2005) Differential location of α-expansin proteins during the accommodation of root cells to an arbuscular mycorrhizal fungus. Planta 220:889–899

Bashline L, Li S, Gu Y (2014) The trafficking of the cellulose synthase complex in higher plants. Ann Bot 114:1059–1067

Bencherif K, Boutekrabt A, Dalpé Y, Lounès-Hadj Sahraoui A (2016) Soil and seasons affect arbuscular mycorrhizal fungi associated with *Tamarix* rhizosphere in arid and semi-arid steppes. Appl Soil Ecol 107:182–190

Bidhendi AJ, Geitmann A (2016) Relating the mechanics of the primary plant cell wall to morphogenesis. J Exp Bot 67:449–461

Boerjan W, Ralph J, Baucher M (2003) Lignin biosynthesis. Annu Rev Plant Biol 54:519–546

Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nat Commun 1:48

Bonfante P, Vian B, Perotto S et al (1990) Cellulose and pectin localization in roots of mycorrhizal *Allium porrum*: labelling continuity between host cell wall and interfacial material. Planta 180:537

Chen XW, Wong JTF, Ng CWW, Wong MH (2016) Feasibility of biochar application on a landfill final cover—a review on balancing ecology and shallow slope stability. Environ Sci Pollut Res 23:7111–7125

Cheng L, Booker FL, Tu C et al (2012) Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO_2. Science 337:1084–1087

Coleman HD, Yan J, Mansfield SD (2009) Sucrose synthase affects carbon partitioning to increase cellulose production and altered cell wall ultrastructure. Proc Natl Acad Sci U S A 106:13118–13123

Cornelissen JHC, Lavorel S, Garnier E et al (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Aust J Bot 51:335–380

Coutinho PM, Deleury E, Davies GJ, Henrissat B (2003) An evolving hierarchical family classification for glycosyltransferases. J Mol Biol 328:307–317

Detering S, Dettmann S, Thierfelder H et al (2005) Glycosidase and glycosyltransferase activity increase in arbuscular mycorrhiza infected legume roots. Symbiosis 40:157–162

Fiorilli V, Catoni M, Miozzi L et al (2009) Global and cell-type gene expression profiles in tomato plants colonized by an arbuscular mycorrhizal fungus. New Phytol 184:975–987

Genet M, Stokes A, Salin F et al (2005) The influence of cellulose content on tensile strength in tree roots. Plant Soil 278:1–9

GEO (Geotechnical Engineering Office, Civil Engineering and Development Department of the Government of the Hong Kong SAR, China) (2000) Guide to Rock and Soil Descriptions. http://www.cedd.gov.hk/eng/publications/geo/doc/eg3.pdf

GEO (Geotechnical Engineering Office, Civil Engineering and Development Department of the Government of the Hong Kong SAR, China) (2011) GEO Publication No. 1/2011. Technical Guidelines on Landscape Treatment for Slopes. http://www.cedd.gov.hk/eng/publications/geo/geo_p111.html. Accessed 21 Aug 2017

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol 84:489–500

Guether M, Balestrini R, Hannah M et al (2009) Genome-wide reprogramming of regulatory networks, transport, cell wall and membrane biogenesis during arbuscular mycorrhizal symbiosis in *Lotus japonicus*. New Phytol 182:200–212

Heimann M, Reichstein M (2008) Terrestrial ecosystem carbon dynamics and climate feedbacks. Nature 451:289–292

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. Calif Agric Exp Stn Circ 347: 1–32

Hoeksema JD, Chaudhary VB, Gehring CA et al (2010) A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecol Lett 13:394–407

Holland N, Holland D, Helentjaris T et al (2000) A comparative analysis of the plant cellulose synthase (CesA) gene family. Plant Physiol 123:1313–1324

Hu W-J, Harding SA, Lung J et al (1999) Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees. Nat Biotechnol 17:808–812

Javot H, Penmetsa RV, Terzaghi N et al (2007) A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. Proc Natl Acad Sci U S A 104:1720–1725

Joshi CP, Mansfield SD (2007) The cellulose paradox — simple molecule, complex biosynthesis. Curr Opin Plant Biol 10: 220–226

Kalia S, Kaith BS, Kaur I (eds) (2011) Cellulose Fibers: Bio- and Nano-Polymer Composites: Green Chemistry and Technology, 2011 edition. Heidelberg, Springer

Karandashov V, Bucher M (2005) Symbiotic phosphate transport in arbuscular mycorrhizas. Trends Plant Sci 10:22–29

Klute A, Weaver RW, Mickelson SH et al (1994) Methods of Soil Analysis: Part 4, Physical Methods. Soil Science Society of America, Madison

Leavitt SW, Danzer SR (1993) Method for batch processing small wood samples to holocellulose for stable-carbon isotope analysis. Anal Chem 65:87–89

Li X, Weng J-K, Chapple C (2008) Improvement of biomass through lignin modification. Plant J 54:569–581

Liu J, Blaylock LA, Endre G et al (2003) Transcript profiling coupled with spatial expression analyses reveals genes involved in distinct developmental stages of an arbuscular mycorrhizal symbiosis. Plant Cell 15:2106–2123

Mayr R, Godoy R (1990) Seasonal patterns in vesicular-arbuscular mycorrhiza in Melic-Beech Forest. Agric Ecosyst Environ 29:281–288

Moran LA, Horton RA, Scrimgeour G, Perry M (2011) Principles of Biochemistry, 5th edn. Prentice Hall, Boston

Nadian H, Smith SE, Alston AM, Murray RS (1996) The effect of soil compaction on growth and P uptake by *Trifolium subterraneum*: interactions with mycorrhizal colonisation. Plant Soil 182:39–49

Nadian H, Smith SE, Alston AM et al (1998) Effects of soil compaction on phosphorus uptake and growth of *Trifolium subterraneum* colonized by four species of vesicular–arbuscular mycorrhizal fungi. New Phytol 140:155–165

Ng CWW, Leung AK, Woon KX (2013) Effects of soil density on grass-induced suction distributions in compacted soil subjected to rainfall. Can Geotech J 51:311–321

Paszkowski U, Kroken S, Roux C, Briggs SP (2002) Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. Proc Natl Acad Sci U S A 99:13324–13329

Pauly M, Gille S, Liu L et al (2013) Hemicellulose biosynthesis. Planta 238:627–642

Pollen N, Simon A (2005) Estimating the mechanical effects of riparian vegetation on stream bank stability using a fiber bundle model. Water Resour Res 41:W07025

Reddy N, Yang Y (2005) Biofibers from agricultural byproducts for industrial applications. Trends Biotechnol 23:22–27

Rich MK, Schorderet M, Reinhardt D (2014) The role of the cell wall compartment in mutualistic symbioses of plants. Front Plant Sci 5:238

Ross P, Mayer R, Benziman M (1991) Cellulose biosynthesis and function in bacteria. Microbiol Rev 55:35–58

Rowell RM (ed) (1984) The Chemistry of Solid Wood, 1st edn. American Chemical Society, Washington, D.C

Saxena IM, Brown RM (2005) Cellulose biosynthesis: current views and evolving concepts. Ann Bot 96:9–21

Scheller HV, Ulvskov P (2010) Hemicelluloses. Annu Rev Plant Biol 61:263–289

Siciliano V, Genre A, Balestrini R et al (2007) Pre-penetration apparatus formation during AM infection is associated with a specific transcriptome response in epidermal cells. Plant Signal Behav 2:533–535

Smith SE, Read DJ (2008) Mycorrhizal Symbiosis, 3rd edn. Academic Press, London

Solaiman ZM (2014) Contribution of Arbuscular Mycorrhizal Fungi to Soil Carbon Sequestration. In: Solaiman ZM, Abbott LK, Varma A (eds) Mycorrhizal Fungi: Use in Sustainable Agriculture and Land Restoration. Springer, Berlin, pp 287–296

Sparks DL, Page AL, Dalton PA et al (1996) Methods of Soil Analysis: Part 3, Chemical Methods. Soil Science Society of America, American Society of Agronomy, Madison

Taylor NG (2008) Cellulose biosynthesis and deposition in higher plants. New Phytol 178:239–252

Tosi M (2007) Root tensile strength relationships and their slope stability implications of three shrub species in the Northern Apennines (Italy). Geomorphology 87:268–283

Treseder KK, Allen MF (2000) Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO_2 and nitrogen deposition. New Phytol 147:189–200

van der Heijden MGA, Klironomos JN, Ursic M et al (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72

Vangelisti A, Natali L, Bernardi R et al (2018) Transcriptome changes induced by arbuscular mycorrhizal fungi in sunflower (*Helianthus annuus* L.) roots. Sci Rep 8:4

Veiga RSL, Faccio A, Genre A et al (2013) Arbuscular mycorrhizal fungi reduce growth and infect roots of the non-host plant *Arabidopsis thaliana*. Plant Cell Environ 36:1926–1937

Watanabe Y, Meents MJ, McDonnell LM et al (2015) Visualization of cellulose synthases in *Arabidopsis* secondary cell walls. Science 350:198–203

Weng J-K, Chapple C (2010) The origin and evolution of lignin biosynthesis. New Phytol 187:273–285

Weng J-K, Li X, Bonawitz ND, Chapple C (2008) Emerging strategies of lignin engineering and degradation for cellulosic biofuel production. Curr Opin Biotechnol 19:166–172

Wightman R, Turner S (2010) Trafficking of the plant cellulose synthase complex. Plant Physiol 153:427–432

Wong CC, Wu SC, Kuek C et al (2007) The role of mycorrhizae associated with vetiver grown in Pb-/Zn-contaminated soils: Greenhouse study. Restor Ecol 15:60–67

Wu TH (2013) Root reinforcement of soil: Review of analytical models, test results, and applications to design. Can Geotech J 50:259–274

Wu TH, McKinnell WP III, Swanston DN (1979) Strength of tree roots and landslides on Prince of Wales Island, Alaska. Can Geotech J 16:19–33

Wu Q-S, Liu C-Y, Zhang D-J et al (2016) Mycorrhiza alters the profile of root hairs in trifoliate orange. Mycorrhiza 26:237–247

Xiao Y, Zhou Q, Shan B (2010) Design and construction of modern bamboo bridges. J Bridg Eng 15:533–541

Xiao C, Zhang T, Zheng Y et al (2016) Xyloglucan deficiency disrupts microtubule stability and cellulose biosynthesis in Arabidopsis, altering cell growth and morphogenesis. Plant Physiol 170:234–249

Youssefian S, Rahbar N (2015) Molecular origin of strength and stiffness in bamboo fibrils. Sci Rep 5:11116

Zeng W, Jiang N, Nadella R et al (2010) A glucurono(arabino)xylan synthase complex from wheat contains members of the GT43, GT47, and GT75 families and functions cooperatively. Plant Physiol 154:78–97

Zhang S-Y, Fei B-H, Yu Y et al (2013) Effect of the amount of lignin on tensile properties of single wood fibers. For Sci Pract 15:56–60

Zhang C-B, Chen L-H, Jiang J (2014) Why fine tree roots are stronger than thicker roots: The role of cellulose and lignin in relation to slope stability. Geomorphology 206:196–202

Zhang Y, Nikolovski N, Sorieul M et al (2016) Golgi-localized STELLO proteins regulate the assembly and trafficking of cellulose synthase complexes in *Arabidopsis*. Nat Commun 7:11656

Zheng Y, Anderson S, Zhang Y, Garavito RM (2011) The structure of sucrose synthase-1 from *Arabidopsis thaliana* and its functional implications. J Biol Chem 286:36108–36118