Effects of Altered Gravity Conditions on Lignin and Secondary Wall Formation in Herbaceous Dicots and Woody Plants

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Abstract

A relationship between lignin formation and gravity is important in biological development of plants in space, because lignin is considered to have played one of the most important roles in the evolution of land plants. This relationship has been studied from a basic biological point of view in herbaceous plants. On the other hand, this relationship is mainly focused on formation of reaction wood in woody plants and has been studied from a practical point of view because of deleterious features of reaction wood for wood manufacture. The International Space Station (ISS) is currently in operation and unique space experiments are to be performed using both herbaceous and woody plants. Recent advances in the study of roles of gravity on lignin and secondary wall formation are reviewed. ©2009 Jpn. Soc. Biol. Sci. Space; Article ID: 092304018

Key words: lignin, gravity, reaction wood, secondary wall, xylem, Arabidopsis

Introduction

The xylem, which has highly lignified secondary cell walls, plays an important role in strengthening plant bodies as well as in transporting water and minerals. Development of xylem has been postulated to play a major role in the evolution of land plants for both physical and physiological reasons, i.e. for resistance to 1 G gravity and for adaptation to the dry environment on land.

Role of gravity in xylem development has been noticed earlier in woody plants than in herbaceous plants. Woody plants have a mechanism for recovering orientation of stems after being bent or tilted out of the vertical (Scurfield, 1973) and thereby directing growing stems toward openings in a forest canopy (Bowyer et al., 2003) by forming a particular cell wall structures called reaction wood. The formation of reaction wood is an inducible process (Cronshaw and Morey, 1965; Wilson and Archer, 1977; Fisher, 1985; Baba et al., 1995). The most important stimulus which are considered to be involved in the formation of reaction wood is gravity and the major alternative stimulus is stress or strain from self weight or external forces (Telewski, 2006; Wilson and Archer, 1977).

Plant cells form two types of cell walls, i.e. primary cell wall and secondary cell wall. The former is first-formed, unspecialized cell wall, which is born in the cell plate during cell division. The latter forms during cell differentiation after cell expansion ceases, which is often multilayered and containing lignin (Taiz and Zeiger, 2002). Lignin is highly branched phenolic polymer made up of phenylpropanoid derivatives that may be associated with celluloses and proteins (Taiz and Zeiger, 2002). In addition to secondary cell walls, primary cell walls in herbaceous plants are also involved in a mechanism to resist gravitational force, recently termed ‘gravity resistance’ (Hoson, 2006), so that they could survive under 1 G environment on Earth. Cellulose microfibrils are embedded in hydrated matrix polysaccharides, that are composed mostly of neutral and acidic polysaccharides, and a small amount of structural proteins in the primary cell wall (Cosgrove, 1999). Growing herbaceous plants resist against gravity by modifying the structure of matrix polysaccharides in primary cell walls (Hoson, 2006).

Lignin commonly found in vascular tissues of herbaceous and woody plants contributes to the strength of cell walls. Gravity has been hypothesized to make cell wall mechanically rigid by enhancing lignification. This hypothesis has been examined under different gravitational forces including microgravity in space. Current advances in the research field related to lignin and gravity in herbaceous dicots and woody plants are reviewed here, while those in gramineous plants is reviewed by Wakabayashi et al. (2009a).

Herbaceous dicots

Independently from a gravity-related research, lignin formation has been paid attention in herbaceous plants from an agronomical viewpoint, because higher lignin content lowers digestibility of forage by livestocks. Genetic manipulation has been attempted to decrease the content of lignin (Anterola and Lewis, 2002). In addition, the secondary growth in herbaceous dicots has been...
Gravity and lignin formation

studied as a model of xylem formation in woody plants. However, even herbaceous dicots actually take a certain period of time to start secondary growth. In Arabidopsis, physiological methods have been used to induce secondary growth in plants of short stature. Therefore, in some cases, effects of gravity on lignin formation have been examined mainly with regard to primary xylem during the period of primary growth.

Although we need to be cautious in interpreting results obtained by the acetyl bromide assay for lignin, this assay has often been used, because it is rapid and sensitive (Hatfield et al., 1999). Hoson et al. (1996) showed that the hypergravity treatment increased the lignin content in cress hypocotyls. Hypergravity treatment significantly increased the content of the secondary cell wall and acetyl bromide-extractable lignins in Arabidopsis inflorescence stems (Tamaoki et al., 2006). Peroxidases have been proposed to catalyze polymerization of monolignols into lignin (Whetten and Sederoff, 1995). Wakabayashi et al. (2009b) reported that hypergravity treatment stimulated the increase in the lignin content and peroxidase activity in the cell wall preparation in azuki bean epicotyls.

On the other hand, controversial results as to lignin formation have been reported in space experiments. Cowles et al. (1984) reported that lignin formation was suppressed under microgravity conditions in mung bean seedlings in space. In shoots and roots of wheat seedlings, there were no significant differences in lignin content between plants grown in space and on Earth (Levine et al., 2001). No differences were observed in lignin content of wheat leaves grown in μ G or 1 G (Stutte et al., 2006). Allen et al. (2009) recently demonstrated that lignin remained constant over the micro- to 4-G range in stems of Brassica rapa. These recent flight experiments may tend to show no significant differences in lignin content, which might be due to well-ventilated systems employed in microgravity experiments, as suggested by Stutte et al. (2006). However, we should put in mind that examined sample sizes have been small in space experiments, and that there still is a possibility of a difference between dicots and monocots that basically lack a capability of secondary growth. In addition, the most scientifically important experimental condition that has not yet been realized in space experiments is that the 1-G control in orbit. Therefore, it is necessary for us to wait the definitive answer until the orbital 1-G control is realized.

It should be noted that careful morphological examinations have been performed for the effects of altered gravity conditions on secondary wall as well. Number of metaxylem elements per xylem, apparent thickness of the secondary thickenings of metaxylem elements, and cross-section area of metaxylem elements in Arabidopsis inflorescence stems increased in response to hypergravity at 300 G for 24 h (Nakabayashi et al., 2006). They also examined mechanical properties of the isolated secondary cell wall preparation and showed that its extensibility decreased in response to hypergravity. Morphometric analysis of Brassica rapa stem tissue confirmed that the areas of pith, cortex, and vascular tissue all increased at 4-G over the 16-d treatment (Allen et al., 2009). Vessel walls of cotyledons were significantly thinner in soybean seedlings grown in space than in 1-G conditions (P< 0.05) and the orientation of cellulose microfibrils, which are composed of 1-4-linked β-D-glucan chains and provide structural integrity to the cell walls, and their assembly in developing vessels were perturbed in space at the beginning of wall deposition (de Micco et al., 2008). More specifically, single cellulose microfibrils were not laid parallel to each other but were spread without any preferential direction in space while they were laid parallel to each other in 1-G conditions (de Micco et al., 2008).

Molecular biological approach has been applied to understanding the mechanism of the effects of altered gravity conditions on lignin and secondary wall. Unique simple experiments were performed in view of the idea that the plant itself carries body weight of a plant imposed by Earth’s gravity. The weight of the inflorescence stem itself has been shown to be responsible for the induction of secondary xylem development in mature Arabidopsis inflorescence stems, because an artificially-applied weight (2.5 g) on the top of the immature inflorescence stem facilitated secondary xylem development (Ko et al., 2004). A custom microarray analysis showed that expressions of 27 of 765 genes related to secondary cell wall formation, such as genes encoding glucanase, laccase, cellulose synthase and peroxidase, were significantly changed in Arabidopsis inflorescence stems after plants were grown on rock wool block until the primary inflorescence stem reached a length of 80–120 mm and then the whole plants were placed horizontally for 30 or 60 min (Yokoyama and Nishitani, 2006). We have recently carried out a comprehensive analysis of gene expression in inflorescence stems of Arabidopsis using microarray (22K) to identify genes whose expression is modulated under hypergravity condition.

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Fig. 1. Schematic diagram showing reaction wood formation in stem looping experiments. (A) Softwood. (B) Hardwood.
**Compression wood**

(300 G) (Tamaoki et al., 2009). They demonstrated that hypergravity up-regulated genes responsible for the biosynthesis or modification of cell wall components such as lignin (putative cinnamate-4-hydroxylase, coumarate 3-hydroxylase, ferulate-5-hydroxylase and peroxidase), xyloglucan, pectin and structural proteins. The three monolignol synthesis-related genes whose expression was up-regulated by hypergravity had been already certified for their involvement in monolignol biosynthesis through characterization of corresponding mutants (Tamaoki et al., 2009).

Regarding the signal transduction for modification of lignin and secondary wall by gravity, mechanosensitve ion channels are suggested to be involved in the perception of gravity stimulus in plants, because Gd ions, known inhibitors of mechanosensors, inhibit the effect of hypergravity on lignin deposition in the secondary cell wall and cell wall extensibility (Tamaoki et al., 2006; Nakabayashi et al., 2006). In addition, a recent study demonstrated that a transient increase in the concentration of cytoplasmic free calcium ions was induced by hypergravity in Arabidopsis seedlings (Toyota et al., 2007).

Participation of plant hormones in signal transduction mechanism has been reported for various responses of plants to altered gravity. A microarray analysis demonstrated that hypergravity at 7 G up-regulates the expression of genes related to plant hormones in Arabidopsis callus culture (Martzigvanou and Hampp, 2003). These studies suggest a possible involvement of plant hormones in signal transduction after perception of the gravity stimulus. Hypergravity at 300 G altered the expression of genes related to the biosynthesis of plant hormones such as auxin and ethylene and that of genes encoding hormone-responsive proteins (Tamaoki et al., 2009), indicating that hypergravity influences the formation of secondary cell walls through modulating the pattern of gene expression, and that auxin and/or ethylene play an important role in signaling of hypergravity stimulus.

**Woody plants**

In the case of secondary growth in woody plants, the gravitropic response includes the formation of reaction wood, which is also a part of ‘gravity resistance’ mechanism. There are different types of reaction wood. Reaction wood formed in gymnosperms is called compression wood, and that formed in angiosperms (hardwood reaction wood) is called tension wood. Research of reaction wood has a long history both in basic plant biology and wood science. This review is just a short glance for that area of research. For details, it should be referred to previous reviews (Timell, 1986; Wilson and Archer, 1977).

**Compression wood**

In gymnosperm trees, such as pine, compression wood forms at the lower side of displaced stems. Compression wood is characterized by short, rounded tracheids that have thick walls with increased lignin content and increased microfibril angles (Timell, 1969). Compression wood also forms almost universally in normal branches of gymnosperm trees (Bowyer et al. 2003). Ultraviolet microspectrophotometry has shown that compressive growth stress is associated with higher lignin content in the secondary wall in compression wood of Cryptomeria japonica (Okuyama et al., 1998).

Both the content of lignin and its monomeric composition of compression wood are quite different from that of opposite wood, which formed on the upper side of branches or displaced stems. In compression wood, secondary xylem tracheids anatomically differ from those of normal wood, and the lignin content is significantly higher, with a large increase in the amount of p-coumaryl alcohol relative to that of coniferyl alcohol (Kwon et al., 2001). In compression wood of Thuja orientalis and Metasequoia glyptostroboides, the activities of enzymes involved in lignin precursor biosynthesis, such as phenylalanine ammonia lyase, 3-O-methyltransferase, p-hydroxycinnamate:CoA ligase and cinnamyl alcohol dehydrogenase, were marginally higher than that of normal wood (Kutsuki and Higuchi, 1982). Following evidence may provide an explanation for the noted increases in the p-coumaryl alcohol content in compression wood lignins. In cell suspension cultures of loblolly pine, Pinus taeda, the ratio of accumulation of coniferyl and p-coumaryl alcohols was about 8:1 when no phenylalanine was added to the medium while that was 1:1, when phenylalanine was added at saturating level (Anterola et al., 1999).

Although the signaling pathway that controls compression wood formation is still poorly understood, ethylene has been reported to be involved in the compression wood formation in Pinus contorta (Savidge, 1983). Interaction between ethylene and IAA in the regulation of compression wood formation was demonstrated by Little and Eklund (1999). Compression wood-responsive proteins were analyzed in developing xylem of Pinus pinaster Ait. The proteins up-regulated in compression wood included an ethylene forming enzyme 1-aminocyclopropane-1-carboxylate oxidase, a putative transcription factor, two genes involved in lignification (caffeic acid O-methyltransferase and caffeoyl CoA-O-methyltransferase), members of the S-adenosyl-L-methionine-synthase gene family (Plomion et al., 2000).

**Tension wood**

Woody angiosperms form tension wood on the upper sides of branches or leaning stems. Tension wood is characterized by a reduction in frequency and size of the vessels, and by an increase in the number of fibers with an inner gelatinous cell wall layer, called G-layer, that is poorly lignified and is composed of cellulose microfibrils that are nearly parallel to the fiber axis (Jourez et al., 2001; Côté et al., 1969). In general, tension wood is characterized by much lower lignin contents (Kucera and Philipson, 1977), although this is
somehow controversial (Timell, 1986). Some primitive angiosperm species lack gelatinous (G-) fibers (Onaka, 1949; Kucera and Philipson, 1977). However, the amount of lignin decreases even in the tension wood region in some Magnolia species that do not form a G-layer in this region. Other anatomical characteristics of reaction wood are variable (Kucera and Philipson, 1977).

In the tension wood of inclined black locust, Robinia pseudoacacia, the G-layer begins to differentiate and its lignin content begins to decrease with increasing tensile growth stress (Yoshida et al., 2002a). Formation of a G-layer, a decrease in lignin and an increase in cellulose microfibrils parallel to the fiber axis in the secondary wall, is indicated to be necessary to produce large tensile growth stress in yellow poplar, Liriodendron tulipifera (Yoshida et al., 2002b).

Regarding the signaling for tension wood formation, asymmetric ethylene production within the poplar stem, which is controlled by ACC oxidase activity, is suggested to be important in the control of tension wood formation in Populus tremula (L.) (Andersson-Gunneras et al., 2003).

**Reaction wood and gravity**

The question what triggers formation of reaction wood is still a matter of debate. Early studies suggested that reaction wood formed as a result of induced compression or tension stress, although this theory was discounted by studies in which young stems were bent into loops (Bowyer et al., 2003). Jaccard (1938) demonstrated that compression wood in the case of softwood formed on the lower side of bottom of the loop where there was tension stress and, therefore, compression wood was not expected to form. The opposite results were obtained in the case of hardwood. These studies indicated that gravity, instead of mechanical stress, played a decisive role in the formation of reaction wood (Bowyer et al., 2003).

Weeping growth of branches of weeping type Japanese cherry is suggested to be caused not by differential growth but by a lack of mechanical rigidity of the branches (Nakamura et al., 1994). Branches of the weeping type Prunus spachiana Kitamura f. spachiana cv. Plenorosea form no tension wood and less xylem than those of the upright type Prunus spachiana Kitamura f. ascendens Kitamura (Baba et al., 1995). These studies indicate that branches lose mechanical rigidity and show weeping growth if reaction wood is not formed. When the woody stem of the upright type Japanese cherry, Prunus jamasakura Siebold ex Koidz., was grown on a three-dimensional clinostat for four weeks, the stem bent during growth, lost its concentric structure of internal tissues, and could not stand straight against gravity after the treatment (Yoneyama et al., 2004). This weakened appearance of the clino-rotated woody stem is somehow similar to a branch of the weeping type. Therefore, these findings together indicate that reaction wood is induced by gravistimulation.

On the other hand, reaction wood was shown to form even in microgravity through mechanical bending. When Douglas fir (Pseudotsuga menziesii) and loblolly pine (Pinus taeda) plants were harnessed, or artificially inclined, at 45 degree to test whether compression wood would be formed in microgravity, all harnessed plants formed compression wood (Kwon et al., 2001). Moreover, not only the cambial cells but also the developing tracheid cells underwent significant morphological changes. It is thus apparent that woody plants can make appropriate corrections to compensate for stress gradients introduced by mechanical bending through compression wood formation even in a microgravity environment (Kwon et al., 2001). These facts again indicate that mechanical stress plays a key role in the formation of reaction wood. Jaccard’s looping experiment is to be performed in the ISS to see whether reaction wood will form on the upper sides of the tops and bottoms of willow stem loops (Savidge, 2008). It is interesting to see whether this experiment will support the result obtained by Kwon et al. (2001).

Gravity appears to play a contradictory role with respect to lignin formation in reaction wood, i.e. gravity increases lignin content in compression wood but decreases it in tension wood. Although it appears still too early to draw conclusions about a common mechanism with regard to the role of gravity on lignin and secondary wall formation in herbaceous dicots and woody plants, focusing on plant hormone signaling may give a clue. Further studies are necessary to understand how the role of gravity has changed during the course of evolution of land plants.

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