Title:

The click-compatible sugar 6-deoxy-alkynyl glucose metabolically incorporates into Arabidopsis root hair tips and arrests their growth

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Graphical Abstract

Arabidopsis root epidermal cells incorporate the click-compatible sugar analog, 6-deoxy-alkynyl glucose, specifically into root hair tip cell walls where it arrests root hair growth.

Abstract

Plant cell walls are dynamic structures whose polysaccharide components are rearranged and recycled during growth and morphogenesis. Covalent fluorescent tagging of these polysaccharides following a metabolic labeling approach can help elucidate these changes. Herein reported are the synthesis and seedling-incorporation of a plant polysaccharide chemical reporter, 6-deoxy-alkynyl glucose (6dAG), that is modeled on D-glucose. Whereas fucose-alkyne, a previously reported chemical reporter for pectin, incorporates diffusely throughout growing cell walls, 6dAG incorporated specifically into root hair tips. This incorporation occurs in a time- and concentration-dependent manner. 6dAG exposure both induces and colocalizes with callose deposition in this tissue, and arrests both root hair and root growth. These results show that plants can incorporate an additional alkynyl-modified sugar analog into their metabolism, and into a discrete subcellular location.

Keywords

click chemistry; *Arabidopsis thaliana*; Brassicaceae; cell wall; root hair; glycan; glucose

1. Introduction

The primary walls of plant cells are dynamic extracellular structures that enable tissue growth and determine cellular and organismal morphology (Cosgrove, 2005). They are composed of carbohydrate polymers that interact with one another to form strong, flexible networks that are reorganized during cell growth (Anderson et al., 2010; Fry, 2004; Somerville et al., 2004). Many of these carbohydrate polymers contain glucose monomers: for example, cellulose, the main load-bearing component of the plant cell wall, is composed of β -1,4-linked glucan chains that coalesce to form partially crystalline microfibrils with high tensile strength (Franks, 2003). Xyloglucan, the most abundant hemicellulose in the primary walls of many eudicot plant species, contains a backbone of β -1,4-linked glucose monomers that is decorated with neutral sugar sidechains, whereas the linear polymer, mixed-linkage glucan, contains β -1,4- and β -1,3-linked glucose subunits. Callose, a specialized cell wall polymer that is produced during new cell wall formation and in response to external stimuli such as wounding and pathogen attack, is composed of linear β -1,3-linked glucan chains (Chen and Kim, 2009; Currier, 1957). Although candidate glycosyltransferases for all of these polymers have been identified at the genetic level, many details of where and when these polymers are synthesized, delivered to the cell wall, and modified during cellular development and expansion are unknown (Lerouxel et al., 2006; Worden et al., 2012). Since cell wall carbohydrates embody much of the biomass produced by plants via photosynthesis, improved knowledge of their dynamics will be useful for the sustainable production of food, materials, and renewable bioenergy.

The ability to specifically label newly synthesized carbohydrate polymers with fluorescent tags is an effective method for following their life histories in living organisms. However, because carbohydrate polymers cannot be tagged genetically in the same way that proteins can be tagged with fluorescent markers such as GFP, they must be labeled by other means. One such approach is metabolic labeling, in which sugar analogs act as chemical reporters, becoming incorporated into natural polymers before being covalently linked to fluorescent probes using, for example, a copper-catalyzed cyclization reaction (a subset of “click reactions”) (Kolb et al., 2001; Laughlin and Bertozzi, 2009). This method has been used to investigate the details of glycan synthesis and metabolism across diverse biological taxa (Beatty et al., 2006; Hsu et al., 2007). For example, a click chemistry-based approach to label nascent pectins in root

epidermal cell walls of the model plant *Arabidopsis thaliana* (L.) Heynh. was previously adopted using the click-compatible fucose analog fucose-alkyne. This study found that fucose-alkyne-containing pectins are initially delivered to discrete locations across the cell surface that likely represent sites of exocytic vesicle fusion and that fucose-alkyne-containing pectins become reorganized into linear fibrillar arrangements as cell elongation progresses and they age in the cell wall (Anderson et al., 2012).

In addition to their reorganization during diffuse growth, cell wall components are also reorganized during the transition to tip growth in the same cell. This transition in wall properties occurs in some *Arabidopsis* root epidermal cells. At a certain point along the root developmental gradient, two distinct cell files become visible, trichoblasts and atrichoblasts, which are distinguishable by the presence of root hair bulges in the former. These files typically alternate along the circumference of the root; their identity is determined by the arrangement of cortex cells below (Dolan et al., 1993). Root hair bulges of trichoblasts are regions of distinct wall biochemistry and mechanics where a subregion of the outer wall changes growth schemes. Unlike the diffuse expansion that had occurred during primary cell expansion, these bulges expand by tip growth, the polarized addition of new wall material to elongate the growing tip (Vissenberg et al., 2001).

Diversifying the click chemistry-based approach to enable the metabolic labeling of cellulose, hemicelluloses, and/or callose would allow for the study of how these polymers change in abundance and distribution over developmental time in any plant species of interest. Although azido-linked analogs for several monosaccharides that are commonly found in cell walls are commercially available, and azido 3-deoxy-D-manno-oct-2-ulosonic acid (KDO-azide) was recently reported to incorporate into pectic rhamnogalacturonan-II (Dumont et al., 2015), it was previously found that fucose-azide is not efficiently incorporated into plant cell walls as compared to fucose-alkyne (Anderson et al., 2012). Thus, there is a need to expand the toolbox of click-compatible analogs of monosaccharides, such as glucose, galactose, xylose, rhamnose, and arabinose, that are present in cell wall carbohydrates. In this work, the synthesis of one such analog, 6-deoxy-alkynyl glucose (**6**) (6dAG), and its use in metabolic labeling experiments in *Arabidopsis* roots, is reported. This compound is incorporated into *Arabidopsis* root epidermal cells in a metabolism-, time-, and concentration-dependent manner, but unexpectedly, 6dAG (**6**) incorporation results in specific labeling of a subcellular structure, the root hair bulge, and inhibits root hair growth, suggesting that it might be incorporated into a novel cell wall component that affects cell wall expansion in root hairs.

2. Results and discussion

2.1. Synthesis and design of 6-deoxy-alkynyl glucose (**6**)

Synthesis of 6dAG started with commercially available D-glucose (**1**) (Fig. 1). To differentiate the C6-OH group from the other hydroxy groups, the C6-OH group was first protected with a *tert*-butyldimethylsilyl (TBS) group following a procedure similar to that reported (Dasgupta and Nitz, 2011), giving intermediate (**2**). The remaining hydroxy groups were protected by methoxymethyl (MOM) groups, which can be easily deprotected under mild acidic conditions and survive the Seyferth–Gilbert homologation required to form the alkyne. The TBS group was removed with tetra-*n*-butylammonium fluoride (TBAF), giving key intermediate (**3**) in high yield. To form an alkyne at the C6-OH group, this carbon was oxidized by Dess–Martin oxidation to an aldehyde, and then the aldehyde was converted into alkyne (**4**) by reacting with diethyl diazomethylphosphonate (Nahrwold et al., 2010). Global deprotection under acidic conditions gave (**5**) in an excellent yield. Acetylation by acetic anhydride gave the corresponding per-*O*-acetylated derivative (**6**). In analogy to fucose-alkyne (Anderson et al., 2012) and because of the acetate's greater utility for biological experiments, (**6**) rather than (**5**) is hereafter referred to as “6dAG” (**6**) for “6-deoxy-alkynyl glucose”.

2.2. Living Arabidopsis seedlings incorporate 6dAG (6) into early differentiation zone epidermal cells

6dAG (6), was next tested as to whether it incorporated into Arabidopsis seedling roots, which have exposed cell walls and are specialized for nutrient absorption. Incorporated alkyne groups were labeled for detection with a click-reaction with azido-functionalized fluorophores (Fig. S1). After incubating seedlings for 24 h in liquid media containing 10 μ M 6dAG, followed by click-reacting with Alexa 594-azide (7), fluorescence was observed in the early differentiation zone of the root (Fig. 2B). Of all the functional groups present in seedling tissue, only cell-incorporated alkyne groups should ligate to azido-functionalized fluorophores, since this reaction is highly selective (Kolb et al., 2001). Alexa 488-alkyne (8) is structurally similar to Alexa 594-azide (7), but it lacks the azide group necessary for an orthogonal reaction with the alkyne group on 6dAG (6). After labeling seedlings incubated with 6dAG (6) for 24 h with Alexa 488-alkyne (8), no significant fluorescence was observed (Fig. 2A). To distinguish between two scenarios, one where active cellular metabolism is necessary for 6dAG (6) incorporation, and another where 6dAG (6) passively adsorbs onto external cell walls, whole seedlings were killed and fixed with 4% paraformaldehyde prior to incubation with 6dAG (6). When first incubated with 6dAG (6), then subjected to click-reaction, pre-fixed seedlings showed only faint autofluorescence at the root tip, indicating that they did not incorporate significant amounts of 6dAG (6) (Fig. 2E). These data support the hypothesis that active metabolism is required for 6dAG (6) incorporation into seedlings. Additionally, peracetylated 6dAG (6), which is more hydrophobic than 5, and should therefore be more membrane-permeable, resulted in greater fluorescence after click-labeling compared to incubation with its non-acetylated counterpart, 5 (Fig. S2), providing further evidence that the chemical reporter must be internalized prior to its incorporation. Likewise, when seedlings were incubated in liquid media lacking 6dAG (6) prior to click-reaction, no Alexa 594-azide (7) fluorescence was observed (Fig. 5E), indicating that exposure to fluorescent dyes alone does not result in dye binding. Only through the combination of active metabolism, 6dAG (6) exposure, and azido-functionalized fluorophore was bright fluorescence observed. 6dAG (6) is a member of a suite of alkynyl-functionalized analogs of glucose, rhamnose, mannose, and sucrose that we have synthesized, of which only 6dAG (6) has so far resulted in significant click-dependent fluorescence after incubation with Arabidopsis seedling roots. Additionally, peracetylated versions of commercially available azido-analogs of glucose (2-deoxy-2-azido-glucose and 6-deoxy-6-azido-glucose) did not result in click-dependent fluorescence when incubated with seedlings (data not shown).

Whether 6dAG (6) is incorporated into cell walls or other cellular compartments was next examined. Click reaction conditions (1 h exposure to Cu(I)) result in cell membrane lysis in elongation zone cells in Arabidopsis seedling roots (Anderson et al., 2012), probably due to Fenton reaction production of hydroxyl radicals, which damage membrane lipids (Kennedy et al., 2011). After subjecting seedlings to click-reaction, it was found that the resulting fluorescence appeared at external cell borders and tips, and that brightfield-visible intracellular bodies exhibited Brownian motion. Without intact membranes, this leaves the cell wall as the most likely compartment that could contain the labeled alkyne groups.

2.3. Seedlings incorporate 6dAG (6) at the tips of growth-arrested root hairs.

After establishing that Arabidopsis seedling roots incorporate 6dAG (6), the specific distribution of this chemical reporter along the developmental gradient embodied in these roots was next examined (Fig. 3). Seedlings incorporate 6dAG (6) at a specific developmental zone, namely, the early differentiation zone where root hair growth initiates (Fig. 3A). 6dAG (6) did not accumulate, however, in the more-rootward elongation and division zones (Fig. 3A), nor did it accumulate in tissues with fully developed root hairs. Furthermore, 6dAG (6) preferentially labeled trichoblasts over atrichoblasts within this zone, localizing to root hair bulges and emergent root hair tips (Fig. 4M). 6dAG (6) patterning varied across this zone, clustering into distinct globules in some root hair bulges (Fig. 3E) and appearing more uniform across the

dome of other bulges (Fig. 3C, D). This labeling pattern was accompanied by a reduction in root hair length (Fig. 2C,F; arrows) that was dose-dependent (Fig. 5A, B). It was also observed that continuous growth on 6dAG (**6**) is toxic to *Arabidopsis* seedlings, as measured by a root elongation assay (Fig. 5C, D). In both cases, addition of excess glucose (**1**) does not ameliorate the toxic effect, though at intermediate concentrations of 6dAG (**6**) (5 μ M, 10 μ M) excess glucose (**1**) does partially attenuate the effect on root elongation. Excess glucose (**1**) resulted in longer roots in most treatments, but at these 6dAG (**6**) concentrations the relative root length elongation due to excess glucose (**1**) was more dramatic (Fig. 5E).

2.4. 6dAG (**6**) incorporation is dose- and time-dependent

Next, the range of concentrations and exposure times that result in detectable incorporation of 6dAG (**6**) was examined. In a 24 h endpoint titration experiment, incubation with concentrations of 6dAG (**6**) as low as 1 μ M resulted in detectable fluorescence, with increasing 6dAG (**6**) concentration resulting in greater fluorescence until saturation near 50 μ M (Fig. 4). Fluorescence was also observed in atrichoblasts, although this fluorescence was saturated after exposure to greater than 5 μ M 6dAG (**6**), and was much dimmer than trichoblast fluorescence (Fig. 4M). Additionally, the time dependence of 6dAG (**6**) incorporation was tested by exposing seedlings to 10 μ M 6dAG (**6**) for 2, 4, 6, 12, 18, and 24 h. If passive adsorption was the mechanism by which 6dAG (**6**) was incorporated into seedlings, this would be expected to occur relatively quickly; however, seedlings required at least 6 hours of exposure to 10 μ M 6dAG (**6**) before this reporter could be detected after click labeling, and 12 hours before fluorescence was significantly higher than background fluorescence (Fig. 6). These data further support the idea that metabolic activity is required for incorporation of 6dAG (**6**) into seedlings. Long liquid-incubation time was not the cause of the fluorescence: seedlings exposed to DMSO for 24 h did not exhibit appreciable fluorescence, whereas seedlings exposed to 10 μ M 6dAG (**6**) for 24 h exhibited bright fluorescence in root hair tips (Fig. 6D,E). These data indicate that 6dAG (**6**) is incorporated into *Arabidopsis* roots in a concentration- and time-dependent manner, with selective accumulation occurring in root hair tips.

2.5. 6dAG (**6**) incorporation sites colocalize with Sirofluor-stained β -1,3 glucans

6dAG (**6**)-dependent fluorescence is associated specifically with the tips of growing root hairs, where cell walls are rapidly synthesized. This suggests that 6dAG (**6**) might be incorporated into the cell wall at the tips of growing root hairs, and the fact that 6dAG (**6**) exposure appears to inhibit root hair elongation implies that it might be incorporated into a polymer that inhibits root hair wall expansion. To begin to address these possibilities, 6dAG (**6**)-treated, click-labeled seedlings were stained with Sirofluor, the purified fluorophore from the dye mixture, Aniline Blue – which fluoresces specifically upon binding β -1,3 glucans (Stone et al., 1984) (Fig. 7). 6dAG (**6**)-exposed seedlings show distinct patches of Sirofluor staining in the same subcellular regions that are click-labeled with 6dAG (**6**) (Fig. 7A-C). Seedlings with no exposure to 6dAG (**6**), but otherwise liquid-incubated, click-reacted, and Sirofluor-stained, showed neither Sirofluor nor 6dAG (**6**)-labeled fluorescence at analogous sites (Fig. 7D-F). This suggests that 6dAG (**6**) both co-localizes with and induces β -1,3 glucan formation at incipient root hair bulges. Sirofluor also stained cells in the division zone of the root tip (Fig. 7A).

2.6. Potential metabolic fates of 6dAG (**6**)

6dAG was synthesized as a part of a suite of click-compatible chemical reporters for biological polysaccharides. A good monosaccharide-mimicking chemical reporter should be accepted by sugar “salvage pathways”, which are enzymatic steps leading from monosaccharides to NDP-monosaccharides, the activated substrates for glycosyltransferases (Bar-Peled and O'Neill, 2011; Feingold, 1982). In this case, 6dAG (**6**) is a molecule that is in theory incompatible with the canonical glucose salvage pathway, in that its modification at the C6 position should block phosphorylation by hexokinase, which is the first

step in activating salvaged glucose (**1**) (Bar-Peled and O'Neill, 2011). While other organisms can activate glucose (**1**) with glucose-1-phosphate phosphodismutase activity (Leloir et al., 1949), no such activity has been found in plants. A search of the MetaCyc database (Caspi et al., 2014) did not reveal any other characterized metabolic steps between glucose (**1**) and glucose-1-phosphate. However, given the reaction mechanism of phosphoglucomutase, which can transfer an enzyme-linked phosphate group to the C1 position and abstract a C6 phosphate (Sutherland et al., 1949), it is possible that 6dAG (**6**) could act as an alternative substrate for this enzyme, which would then be converted to its respective phosphate, then UDP-esters. Alternatively, the alkynyl group on 6dAG (**6**) could mimic a relatively hydrophobic aglycone of a small C-glycosyl compound. In this scenario, incorporation could proceed through the action of an enzyme acting on such a compound and transferring the sugar moiety to a polysaccharide acceptor.

Despite not having a clear metabolic route to polysaccharide incorporation, 6dAG (**6**) could be incorporated into a variety of cell wall polymer destinations if it does, in fact, behave as a glucose (**1**) analog. Callose, a β -1,3 glucan, is a possible molecule into which 6dAG (**6**) could incorporate. Xyloglucan and cellulose both have β -1,4 linked glucose backbones, but most xyloglucan glucose residues are decorated at C6 with xylose or a xylose-containing oligomer. This position is unavailable for xylose binding in 6dAG (**6**). Cellulose is unlikely to accommodate 6dAG (**6**), since the orientation at C6 determines its crystal structure and replacement at this position of an alkynyl group would have drastic consequences for crystallization (Nelson and O'Connor, 1964). It is also possible that 6dAG (**6**) is polymerized into a novel glucan analog. Cell wall matrix polysaccharides, with the exception of callose (Hong et al., 2001), are synthesized by glycosyltransferases in the Golgi. Specific glycosyltransferases are uniquely required for tip-growth in plant cells, e.g. *CELLULOSE SYNTHASE-LIKE D (CSLD)* family genes show highly-upregulated expression in pollen tubes and root hair tips (Bernal et al., 2008), and *CSLD3* is required both for the normal organization and possibly biosynthesis of a cellulose-like β -1,4 glucan in root hair cell walls (Galway et al., 2011; Park et al., 2011) and for the initiation of root hair growth (Favery et al., 2001). Biochemical activities unique to the emergent root hair tip may predispose this region to incorporate 6dAG (**6**) here and not in other plant cell walls. Alternatively, a glycosylhydrolase in the apoplast might be able to act in reverse to add 6dAG (**6**) to existing wall polymers. However, the finding that peracetylated 6dAG (**6**) is more efficiently incorporated into plant tissues than its non-acetylated precursor **5** (Fig. S2) does not support this hypothesis, since these data suggest that the entry of 6dAG (**6**) into the cell is important for its incorporation.

Callose is a known component of nascent cell walls; its deposition timing is essential for cytokinesis and pathogen/wounding responses (Thiele et al., 2009). Callose is deposited during normal tip growth of pollen tubes (Chebli et al., 2012), and as an abiotic and biotic stress response in root hair tips (Cooper, 1982; Galway et al., 2011). 6dAG (**6**) colocalizes with callose (Fig. 7), and it may induce callose formation by eliciting callose synthesis as a wounding response due to 6dAG (**6**) toxicity. In addition, 6dAG (**6**) could incorporate into the toxin-induced callose in root hairs. If 6dAG (**6**) is behaving as a glucose (**1**) analog, it might be incorporated into either the poorly-understood β -1,4 glucan deposited by *CSLD3* at root hair tips, or into toxicity-induced callose. Further analysis of 6dAG (**6**)-labeled seedlings, including cell wall fractionation and enzymatic digestion, will be required to pinpoint the identity of the polymer into which 6dAG (**6**) is incorporated.

2.7. 6dAG (6**) incorporation differs markedly from FucAl incorporation**

The two other click-compatible sugar analogs that have been reported to incorporate into plant cell walls thus far are fucose-alkyne, which is thought to label a pectic polysaccharide (Anderson et al., 2012), and KDO-azide, which incorporates into rhamnogalacturonan-II (Dumont et al., 2015). 6dAG (**6**) behaves very differently from fucose-alkyne and KDO-azide: Arabidopsis root epidermal cells incorporate these reporters in as little as 30 minutes, whereas appreciable 6dAG (**6**) incorporation occurs only after several hours (Fig. 6). Whereas the substitution of an alkyne group for the C6 methyl group of fucose is

apparently tolerated by fucose salvage pathway enzymes, the more drastic substitution for a hydroxy group in the case of 6dAG (**6**) eliminates most of the potential salvage and activation metabolic routes for this compound. Fucose-alkyne is not uniformly distributed after short incorporation periods, but it does eventually become incorporated throughout the cell wall (Anderson et al., 2012), as does KDO-azide (Dumont et al., 2015) whereas 6dAG (**6**) is preferentially localized to root hair primordia, a specific sub-region of the wall, where it accumulates. These data suggest that pectin is delivered evenly across the growing cell wall, whereas the polymer into which 6dAG (**6**) is incorporated is apparently targeted specifically to root hair tips. This could be accomplished by a polarized vesicle trafficking pathway or perhaps a root hair-specific glycosyltransferase (Park et al., 2011).

3. Conclusion

6dAG (**6**) has the potential to probe glycan metabolism in many different biological systems, including plants. Click-chemistry is one of few methods compatible with *in vivo* imaging of polysaccharides, compounds that are otherwise difficult to label (Sletten and Bertozzi, 2009). The toolbox of click-compatible sugar analogs relevant to plant cell wall chemistry is currently restricted to fucose-alkyne and KDO-azide (Dumont et al., 2012; Dumont et al., 2015). Synthesis of 6dAG (**6**) and the finding that it incorporates into *Arabidopsis* seedlings in a specific manner add this compound to a short but growing list of chemical reporters available for click-powered glycobiology in plants. Future studies will investigate whether these probes are suitable for probing cell wall dynamics in a wide variety of plant tissues and species.

4. Experimental

4.1 Seedling culture

All click labeled specimens were 4 d *Arabidopsis thaliana* Col-0 seedlings. 30% (v/v) NaOCl surface-sterilized seeds were imbibed for at least 3 days, then grown vertically for 4 d on MS plates (0.8% agar-agar, MS salts and vitamins used at half the strength as in (Murashige and Skoog, 1962)) at constant light, 22 °C. 6dAG (**6**) root elongation assays were performed on vertical MS plates to which filter-sterilized 6dAG in DMSO and/or filter-sterilized glucose (**1**) in H₂O was added during agar cooling.

4.2. 6-deoxy-alkynyl glucose (**6**) click-labeling

Seedlings were click-labeled in a two-step process: (1) incubation with 6dAG (**6**), (2) click-reaction with a click-compatible fluorophore. Seedlings were transferred from plates, washed with liquid MS (growth media lacking agar-agar), then incubated in liquid MS (1.8 mL) containing 10 µM 6dAG (**6**) for 24 h. 6dAG (**6**) was supplied from a 10 mM stock solution in DMSO. After 24 h incubation at 22 °C, seedlings were washed four times with liquid MS, then transferred to a click-reaction solution, containing 1 mM CuSO₄, 1 mM ascorbic acid, and 1 µM azido-functionalized Alexa fluorophore (Alexa594-azide (**7**) for most experiments, Alexa488-azide (**9**) for titration experiment). Ascorbic acid and CuSO₄ were introduced from 100 mM stock solutions in H₂O; ascorbic acid stock solutions were made less than 30 minutes before use. The labeling reaction proceeded in the dark for 1 h at 25 °C, and was quenched by washing four times with liquid MS. Labeled seedlings were stored at 4 °C for less than 48 h before imaging. Seedlings counterstained with Sirofluor were stained for 30 min with 0.01% (w/v) dye solution in H₂O, then washed with H₂O

4.3. Microscopy and image analysis

Root tips of seedlings were imaged with a Zeiss Cell Observer SD spinning disk confocal microscope (488 nm laser excitation, 525/25 emission filter; 561 nm laser excitation, 617/73 emission filter) using either a 63X 1.40 NA oil immersion objective or a 10X 0.3 NA air objective. Z-stacks through the proximal layer of epidermal cells (63X) or half the diameter of the entire root (10X) were collected.

Cell wall-associated fluorescence intensity in maximum projections for 63X images was measured using ImageJ, by drawing circular regions of interest at the rootward end of epidermal cells. Fluorescence intensity for 10X images was calculated by thresholding every maximum projection image above a constant pixel intensity threshold, and then calculating the raw integrated pixel intensity of the remaining image. The threshold was chosen to exclude background, but include globular fluorescent regions at the rootward end of trichoblasts.

Root and root hair lengths were measured from images with the aid of ImageJ software. Images of seedlings growing on agar plates were taken on successive days with a flatbed scanner, from which root lengths were traced and measured. 10X micrographs of root hairs were stitched together with the MosaicJ plugin for ImageJ (Thevenaz and Unser, 2007). Root hair lengths were measured by tracing hairs; root hair distance along the root axis was measured by finding the point on a traced root axis that was closest to the root hair base, and measuring the distance along the axis from the root tip to that point.

4.4. Synthesis of 6-deoxy-alkynyl glucose (6)

4.4.1. Synthesis of 6-TBDMS-D-glucose (2)

To an ice-water cooled solution of D-glucose (**1**) (5.4 g, 30 mmol, 1 equiv) in dry pyridine (50 mL) was added *tert*-butyldimethylsilyl chloride (5.4 g, 36 mmol, 1.2 equiv) portionwise. The ice-water bath was removed after addition, and the reaction mixture was stirred at room temperature overnight. The solution was diluted with EtOAc (250 mL) and washed with diluted HCl and brine. The residue after concentration of the organic layer was purified on flash silica gel chromatography using CH₂Cl₂ : MeOH (15 : 1), giving 6-TBDMS-D-glucose (**2**) (4.1 g) as a white foam in a yield of 46%. Compound **2** was a mixture of α - and β - anomers, which were inseparable. ¹H NMR (MeOH-d₄, 360 MHz, ppm): δ 0.08 (s, 6H), 0.91 (s, 9H), 3.09-3.11 (m, 0.45 H), 3.31-3.37 (m, 4H, solvent residue peak included), 3.64-3.69 (t, *J* = 9.0, 9.4 Hz, 0.57 H), 3.74-3.85 (m, 2H), 3.93-3.97 (d, *J* = 11.1 Hz, 0.50 H), 4.43-4.45 (d, *J* = 7.6 Hz, 0.46 H), 5.08-5.09 (d, *J* = 3.2 Hz, 0.49 H); ¹³C NMR (MeOH-d₄, 90 MHz, ppm) δ -5.14, -5.02, 19.34, 26.45, 64.13, 64.30, 71.50, 71.56, 73.20, 73.84, 74.91, 76.23, 78.23, 93.92, 98.12, 101.37; HRMS: calculated for C₁₂H₂₆NaO₆Si⁺ [M+Na⁺]: 317.1391; found: 317.1392.

4.4.2. Synthesis of 1,2,3,4-tetra-*O*-methoxymethyl-D-glucose (3)

To an ice-water cooled solution of 6-TBDMS-D-glucose (**2**) (4.0 g, 13.6 mmol, 1 equiv) in dry DMF (50 mL) was carefully added NaH (3.3 g, 81.5 mmol, 6 equiv, 60% suspended in mineral oil). After 15 minutes, methoxymethyl chloride (5.2 mL, 68.0 mmol, 5 equiv) in DMF (10 mL) was added dropwise to the solution. The ice-water bath was removed after addition, and the reaction mixture was stirred at room temperature for 2 h. H₂O (100 mL) was slowly added to the reaction mixture to quench the reaction. The resulting clear solution was extracted with EtOAc (50 mL) three times. The combined organic layers were washed thoroughly with water and brine, and dried (anhydr. Na₂SO₄). The residue after concentration was used for the next step without further purification.

To a solution of the residue in tetrahydrofuran (50 mL) was added tetra-*n*-butylammonium fluoride (5.3 g, 20.4 mmol, 1.5 equiv). The reaction mixture was stirred at room temperature for 2 h. After completion, the reaction was diluted with EtOAc (200 mL) and washed thoroughly with H₂O and brine. The residue

after concentration of the organic layer was purified on flash silica gel chromatography using hexanes : EtOAc (3 : 1), giving 1,2,3,4-tetra-*O*-methoxymethyl-D-glucose (**3**) (4.0 g) as a white foam in a yield of 82% over two steps. Compound **3** was a mixture of α - and β - anomer, which were inseparable. ¹H NMR (CDCl₃, 360 MHz, ppm): δ 2.72-2.76 (m, 1 H), 3.27-3.38 (m, 13 H), 3.52-3.63 (m, 2 H), 3.76-3.78 (m, 1.8 H), 4.52-4.56 (m, 1.6 H), 4.65-4.77 (m, 3.2 H), 4.83-4.87 (m, 2.8 H), 4.93-4.95 (m, 1 H); ¹³C NMR (CDCl₃, 90 MHz, ppm) δ 55.66, 56.11, 56.18, 56.30, 56.38, 56.46, 61.59, 71.34, 75.17, 76.36, 76.57, 76.80, 77.16, 77.36, 77.51, 77.61, 77.94, 78.24, 80.91, 93.60, 94.83, 97.10, 97.42, 98.22, 98.41, 98.93; HRMS: calculated for C₁₄H₂₈NaO₁₀⁺ [M+Na⁺]: 379.1575; found: 379.1581.

4.4.3. Synthesis of 1,2,3,4-tetra-*O*-methoxymethyl-D-glucose-6-alkyne (**4**)

To an ice-water cooled solution of compound **3** (4.0 g, 11.2 mmol, 1 equiv) in CH₂Cl₂ (100 mL) was added Dess-Martin periodinane (7.1 g, 16.8 mmol, 1.5 equiv). The reaction mixture was stirred at room temperature for 5 h. After completion, the milky solution was washed thoroughly with saturated Na₂S₂O₃ solution and brine. The organic layer was dried (anhydr. Na₂SO₄) and concentrated. The residue was used for the next step without further purification.

A solution of diethyl diazomethylphosphonate (2.0 g, 11.2 mmol, 1 equiv) and the residue obtained above in tetrahydrofuran (50 mL) was cooled in an ice-water bath. NaH (1.1 g, 28 mmol, 2.5 equiv, 60% suspended in mineral oil) was added portionwise in an Ar counterflow. The cooling bath was removed after addition. The reaction mixture was stirred for 30 minutes, and subsequently quenched by careful addition of H₂O (100 mL). The obtained mixture was extracted with EtOAc (50 mL) twice. Combined organic layers were dried (anhydr. Na₂SO₄) and concentrated *in vacuo*. Purification of the residue by flash silica gel chromatography using hexanes : EtOAc (3 : 1) 1,2,3,4-tetra-*O*-methoxymethyl-D-glucose-6-alkyne (**4**) (2.9 g) as a yellow syrup in a yield of 74% over two steps. Compound **4** was a mixture of α - and β - anomers, which were inseparable. ¹H NMR (CDCl₃, 360 MHz, ppm): δ 2.46-2.49 (m, 1.5 H), 3.35-3.67 (m, 23.5 H), 3.85-3.90 (m, 0.5 H), 4.02-4.06 (m, 1H), 4.38-4.41 (m, 0.5 H), 4.56-5.13 (m, 15 H); ¹³C NMR (CDCl₃, 90 MHz, ppm) δ 55.79, 56.27, 56.37, 56.44, 56.49, 56.57, 56.81, 56.85, 62.34, 66.10, 74.15, 74.58, 78.40, 78.71, 79.69, 80.09, 80.82, 93.61, 93.97, 94.89, 97.31, 97.51, 97.84, 98.39, 98.45; HRMS: calculated for C₁₅H₂₆NaO₉⁺ [M+Na⁺]: 373.1469; found: 374.1467.

4.4.4. Synthesis of glucose-6-alkyne (**5**)

Compound **4** (2.5 g, 7.1 mmol, 1 equiv) was suspended in 0.1% H₂SO₄ (50 mL). The resulting solution was heated at 80 °C overnight. Following neutralization with 1 M NaHCO₃, the solution was concentrated to dryness. Purification of the residue by flash silica gel chromatography using CH₃Cl : MeOH (10 : 1) gave 1.1 g of glucose-6-alkyne (**5**) (1.1 g) as a white solid in a yield of 89%. Glucose-6-alkyne (**5**) was an inseparable mixture of α - and β - anomers, with a ratio of 1:0.7 determined by proton NMR. ¹H NMR (MeOH-d₄, 400 MHz, ppm): δ 2.74-2.75 (d, *J* = 1.8 Hz, 1H), 2.82-2.83 (d, *J* = 1.8 Hz, 0.62 H), 3.08-3.12 (t, *J* = 8.4, 8.4 Hz, 0.7 H), 3.23-3.34 (m, 8H), 3.54-3.58 (t, *J* = 9.2, 9.2 Hz, 1H), 3.92-3.94 (d, *J* = 7.6 Hz, 0.7 H), 4.39-4.43 (m, 1.7 H), 5.02-5.03 (d, *J* = 3.6 Hz, 1H); ¹³C NMR (MeOH-d₄, 100 MHz, ppm) δ 62.41, 67.01, 72.14, 72.87, 73.09, 73.76, 73.94, 74.26, 74.52, 75.89, 80.02, 81.06, 92.71, 96.83; HRMS: calculated for C₇H₉O₅⁻ [M-H⁺]: 173.0455; found: 173.0464.

4.4.5. Synthesis of glucose-6-alkyne tetraacetate (**6**) (“6dAG”)

To an ice-water cooled solution of glucose-6-alkyne (**5**) (174 mg, 1 mmol, 1 equiv) in pyridine (5 mL) was added Ac₂O (942 μ L, 10 mmol, 10 equiv), and the reaction was stirred at room temperature overnight. MeOH (1 mL) was added to quench the reaction. The solution was concentrated *in vacuo*, and the residue was purified by flash silica gel chromatography using hexanes : EtOAc (2 : 1), giving glucose-6-alkyne tetraacetate (**6**) (300 mg) as a white solid in a yield of 88%. Glucose-6-alkyne tetraacetate (**6**) was an inseparable mixture of α - and β - anomers, with a ratio of 1:0.5 determined by proton NMR. ¹H

NMR (CDCl₃, 360 MHz, ppm): δ 2.03-2.20 (m, 19 H), 2.51-2.55 (m, 1.3 H), 4.38-4.40 (m, 0.5H), 4.65-4.68 (m, 1H), 5.10-5.25 (m, 3.4 H), 5.40-5.46 (m, 1H), 5.72-5.75 (d, J = 7.9 Hz, 0.5 H), 6.35-6.36 (d, J = 3.6 Hz, 1H); **¹³C NMR** (CDCl₃, 90 MHz, ppm) δ 20.52, 20.67, 20.75, 20.85, 20.93, 62.80, 65.42, 69.07, 69.26, 70.09, 70.91, 72.05, 75.63, 75.99, 88.75, 91.41, 168.61, 168.92, 169.29, 169.65, 170.08, 170.19; **HRMS**: calculated for C₁₅H₁₈NaO₉⁻ [M-H⁺]: 365.0843; found: 365.0840.

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Figure Legends

Figure 1: Synthesis scheme for 6-deoxy-alkynyl glucose (6). Reagents and conditions: a) TBSCl, pyridine, overnight, 46%; b) MOMCl, NaH, DMF, 2 h then TBAF, THF, 2 h, 82% over two steps; c) DMP, DCM, 5 h then diethyl diazomethylphosphonate, NaH, THF, 0.5 h, 74% over two steps; d) 0.1% H₂SO₄, 80 °C, overnight, 89%; e) Ac₂O, pyridine, overnight, 88%.

Figure 2. Arabidopsis seedlings metabolically incorporate exogenous 6-deoxy-alkynyl glucose (6). Representative maximum projections of z series of living (A-C) or paraformaldehyde-fixed (D-F) seedling root tips incubated with 10 μ M 6dAG (6) in liquid MS for 24 h, followed by click-reaction with Alexa 488-alkyne (8) (A,D) and Alexa 594-azide (7) (B,E). Brightness and contrast settings are identical for (A,D) and (B,E). Brightfield images of seedlings are shown in (C,F). Arrowhead and arrow indicate stunted and control root hairs, respectively. Scale bar = 100 μ m.

Figure 3. Arabidopsis seedlings incorporate 6-deoxy-alkynyl glucose (6) into developing root hair bulges and tips in the early differentiation zone. (A) Low-magnification mosaic maximum projection of root tip of an Arabidopsis seedling incubated 10 μ M 6dAG (6) in liquid MS for 24 h, followed by click-reaction with Alexa 594-azide (7). (B) Mosaic maximum projection of the early differentiation zone of an Arabidopsis root incubated with 10 μ M 6dAG (6) in liquid MS for 24 h, followed by click-reaction with Alexa 594-azide (7). Root tip is oriented to the lower left. (C) Overlay of Alexa 594-azide (7) fluorescence (red) and brightfield. (D-F) Detail of older (D,E) and newly forming (F) root hair bulges labeled with Alexa 594-azide (7). Scale bars = 50 μ m.

Figure 4. Fluorescence associated with 6-deoxy-alkynyl glucose (6) incorporation is concentration-dependent. (A-F) Elongation-zone root epidermal cells after 24 h incubation with increasing concentrations (A = 0 μ M; B = 1 μ M; C = 5 μ M; D = 10 μ M; E = 50 μ M) of 6dAG (6), followed by click-reaction with Alexa 488-azide (9). (F) Fluorescence after coincubation with 50 μ M 6dAG (6) and 5 mM glucose (1). Root tip is oriented to the lower left. Pixel intensities are depicted with equivalent brightness and contrast settings between images. (G-L) A-F with pixel intensities transformed with a gamma correction function (γ = 0.4) to show greater detail in darker regions. Note extended root hairs in (G-I) and lack of root hair growth in (J-L). (M) Mean seedling fluorescence intensity for regions of interest at the rootward end of cells in trichoblast cell files (blue) and atrichoblast cell files (red). Open symbols denote incubation with 6dAG (6); filled symbols denote coincubation with 6dAG (6) and 5 mM glucose (1). See (J) for example regions of interest. Error bars are $\pm$ 95% CI, n = 15 - 17 seedlings per treatment. Scale bar = 50 μ m.

Figure 5. 6-deoxy-alkynyl glucose (6) inhibits root and root hair elongation, regardless of the presence of excess glucose (1). (A) Representative brightfield mosaic images of primary roots from seedlings incubated in liquid MS containing varying [6dAG (6)] and [glucose (1)]. Scale bar = 100 μ m. (B) Root hair lengths, plotted along their developmental gradient. Distances were measured from the root hair bases' nearest point on the root axis to the root tip, along the root axis. 16-73 root hair length measurements from each of 6-8 seedlings per [6dAG (6)] exposure level were pooled by treatment, then fit with a Loess best-fit curve. Error shading = $\pm$ 95% CI. (C) Representative images of seedlings grown vertically for 8 d on agar containing varying [6dAG (6)], with and without excess glucose (1) (1 mM). Scale bar = 1 cm. (D) Primary root growth curves for vertically-grown seedlings on agar plates doped with 6dAG (6) and/or excess glucose (1). One representative replicate of three experiments is shown. Error bars are $\pm$ 95% CI; n = 20-36 seedlings per treatment. (E) Glucose (1) enhancement of root elongation, reported as a ratio between lengths of primary roots grown with and without excess glucose (1). Error bars are $\pm$ 95% CI.

Figure 6. 6-deoxy-alkynyl glucose (6) incorporation is time-dependent. (A-D) Elongation-zone root epidermal cells exposed to 10 μ M 6dAG (6) for varying incubation time, followed by click-reaction with Alexa 594-azide (7). (E) Control seedling exposed to DMSO (6dAG (6) solvent) for 24 h. Pixel intensities are depicted with equivalent brightness and contrast settings between images. (F) Summed fluorescence intensity for pixels above a thresholded value for images of seedlings with increasing 6dAG (6) incubation time (filled symbols) or an equivalent volume of DMSO (open symbol). Note that for 2 h and 4 h incubations, fluorescence images are not shown. Error bars are $\pm$ 95% CI, n = 9 seedlings per treatment. Scale bar = 100 μ m.

Figure 7. 6-deoxy-alkynyl glucose (6) colocalizes with and induces β -1,3-glucan staining at root hair bulges. (A-C) Representative seedling treated with 10 μ M 6dAG (6) for 12 h, followed by click-reaction with Alexa 594-azide, Sirofluor staining. (D-F) Seedling treated with DMSO for 12 h followed by click-reaction with Alexa 594-azide, Sirofluor staining. Alexa 594-azide (7) (A,D) is magenta in (C,F); Sirofluor (B,E) is green in (C,F). Identical brightness and contrast adjustments between treatments were made for each channel. Scale bar = 100 μ m.

Figure S1. Structures of fluorescent probes used in this study

The following click-compatible fluorescent probes: Alexa 594-azide (7), Alexa 488-alkyne (8), and Alexa 488-azide (9) were used in this study.

Figure S2. Acetylated 6dAG (6) has enhanced incorporation versus its deacetylated precursor (5). Early differentiation zone root epidermal cells of: (A) Seedling treated with 10 μ M 6dAG (6) for 24 h, followed by click-reaction with Alexa 488-azide (9) and Sirofluor staining. (B) Seedling treated with 10 μ M intermediate 5 for 24 h, followed by click-reaction with Alexa 488-azide (9). (C) Same seedling as (A), but imaged with 100-fold less detector gain. (D) Seedling treated with DMSO for 24 h, followed by click-reaction with Alexa 488-azide (9). Exposure settings, and brightness and contrast adjustments are identical for images A, B, and D. Scale bar = 10 μ m.

References

- Anderson, C. T., Carroll, A., Akhmetova, L., Somerville, C., 2010. Real-time imaging of cellulose reorientation during cell wall expansion in Arabidopsis roots. *Plant Phys.* 152, 787-796.
- Anderson, C. T., Wallace, I. S., Somerville, C. R., 2012. Metabolic click-labeling with a fucose analog reveals pectin delivery, architecture, and dynamics in Arabidopsis cell walls. *Proc. Natl. Acad. Sci. U.S.A.* 109, 1329-1334.
- Bar-Peled, M., O'Neill, M. A., 2011. Plant nucleotide sugar formation, interconversion, and salvage by sugar recycling. *Annu. Rev. Plant Biol.* 62, 127-155.
- Beatty, K. E., Liu, J. C., Xie, F., Dieterich, D. C., Schuman, E. M., Wang, Q., Tirrell, D. A., 2006. Fluorescence visualization of newly synthesized proteins in mammalian cells. *Angew. Chem. Int. Ed.* 45, 7364-7367.
- Bernal, A. J., Yoo, C. M., Mutwil, M., Jensen, J. K., Hou, G., Blaukopf, C., Sorensen, I., Blancaflor, E. B., Scheller, H. V., Willats, W. G. T., 2008. Functional analysis of the cellulose synthase-like genes CSLD1, CSLD2, and CSLD4 in tip-growing Arabidopsis Cells. *Plant Physiol.* 148, 1238-1253.
- Caspi, R., Altman, T., Billington, R., Dreher, K., Foerster, H., Fulcher, C. A., Holland, T. A., Keseler, I. M., Kothari, A., Kubo, A., Krummenacker, M., Latendresse, M., Mueller, L. A., Ong, Q., Paley, S., Subhraveti, P., Weaver, D. S., Weerasinghe, D., Zhang, P. F., Karp, P. D., 2014. The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of Pathway/Genome Databases. *Nucleic Acids Res.* 42, D459-D471.
- Chebli, Y., Kaneda, M., Zerzour, R., Geitmann, A., 2012. The cell wall of the Arabidopsis pollen tube-spatial distribution, recycling, and network formation of polysaccharides. *Plant Physiol.* 160, 1940-1955.
- Chen, X. Y., Kim, J. Y., 2009. Callose synthesis in higher plants. *Plant Signal. Behav.* 4, 489-492.
- Cooper, K. M., 1982. Callose-deposit formation in radish root hairs. In: Brown, R. M. (Ed.), *Cellulose and Other Natural Polymer Systems*. Plenum Press, New York, pp. 167-184.
- Cosgrove, D. J., 2005. Growth of the plant cell wall. *Nat. Rev. Mol. Cell Biol.* 6, 850-861.
- Currier, H. B., 1957. Callose substance in plant cells. *Am. J. Bot.* 44, 478-488.
- Dasgupta, S., Nitz, M., 2011. Use of N,O-dimethylhydroxylamine as an anomeric protecting group in carbohydrate synthesis. *J. Org. Chem.* 76, 1918-1921.
- Dolan, L., Janmaat, K., Willemsen, V., Linstead, P., Poethig, S., Roberts, K., Scheres, B., 1993. Cellular organisation of the *Arabidopsis thaliana* root. *Development* 119, 71-84.
- Dumont, A., Malleron, A., Awwad, M., Dukan, S., Vauzeilles, B., 2012. Click-mediated labeling of bacterial membranes through metabolic modification of the lipopolysaccharide inner core. *Angew. Chem. Int. Ed.* 51, 3143-3146.
- Dumont, M., Lehner, A., Vauzeilles, B., Malassis, J., Marchant, A., Smyth, K., Linclau, B., Baron, A., Mas Pons, J., Anderson, C. T., Schapman, D., Galas, L., Mollet, J. C., Lerouge, P., 2015. Plant cell wall imaging by metabolic click-mediated labelling of rhamnogalacturonan II using azido 3-deoxy-D-manno-oct-2-ulosonic acid. *Plant J.* epub Dec 16.
- Favery, B., Ryan, E., Foreman, J., Linstead, P., Boudonck, K., Steer, M., Shaw, P., Dolan, L., 2001. KOJAK encodes a cellulose synthase-like protein required for root hair cell morphogenesis in Arabidopsis. *Genes Dev.* 15, 79-89.
- Feingold, D. S., 1982. Aldo (and keto) hexoses and uronic acids. In: Loewus, F. A., Tanner, W. (Eds.), *Plant Carbohydrates I: Intracellular Carbohydrates*, vol. 13A. Springer, Würzburg, pp. 3-76.
- Franks, P. J., 2003. Use of the pressure probe in studies of stomatal function. *J. Exp. Bot.* 54, 1495-1504.
- Fry, S. C., 2004. Primary cell wall metabolism: tracking the careers of wall polymers in living plant cells. *New Phytol.* 161, 641-675.
- Galway, M. E., Eng, R. C., Schiefelbein, J. W., Wasteneys, G. O., 2011. Root hair-specific disruption of cellulose and xyloglucan in AtCSLD3 mutants, and factors affecting the post-rupture resumption of mutant root hair growth. *Planta* 233, 985-999.
- Hong, Z., Delauney, A. J., Verma, D. P., 2001. A cell plate-specific callose synthase and its interaction with phragmoplastin. *Plant Cell* 13, 755-768.
- Hsu, T. L., Hanson, S. R., Kishikawa, K., Wang, S. K., Sawa, M., Wong, C. H., 2007. Alkynyl sugar analogs for the labeling and visualization of glycoconjugates in cells. *Proc. Natl. Acad. Sci. U.S.A.* 104, 2614-2619.

Kennedy, D. C., McKay, C. S., Legault, M. C. B., Danielson, D. C., Blake, J. A., Pegoraro, A. F., Stelow, A., Mester, Z., Pezacki, J. P., 2011. Cellular consequences of copper complexes used to catalyze bioorthogonal click reactions. *J. Am. Chem. Soc.* 133, 17993-18001.

Kolb, H. C., Finn, M. G., Sharpless, K. B., 2001. Click chemistry: diverse chemical function from a few good reactions. *Angew. Chem. Int. Ed.* 40, 2004-2021.

Laughlin, S. T., Bertozzi, C. R., 2009. Imaging the glycome. *Proc. Natl. Acad. Sci. U.S.A.* 106, 12-17.

Leloir, L. F., Trucco, R. E., et al., 1949. The formation of glucose diphosphate by *Escherichia coli*. *Arch. Biochem.* 24, 65-74.

Lerouxel, O., Cavalier, D. M., Liepman, A. H., Keegstra, K., 2006. Biosynthesis of plant cell wall polysaccharides - a complex process. *Curr. Opin. Plant Biol.* 9, 621-630.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant.* 15, 473-497.

Nahrwold, M., Bogner, T., Eissler, S., Verma, S., Sewald, N., 2010. "Clicktophycin-52": A bioactive cryptophycin-52 triazole analogue. *Org. Lett.* 12, 1064-1067.

Nelson, M. T., O'Connor, R. T., 1964. Relation of certain infrared bands to cellulose crystallinity and crystal lattice type. part I. spectra of lattice types I, II, III and of amorphous cellulose. *J. Appl. Polym. Sci.* 8, 1311-1324.

Park, S., Szumlanski, A. L., Gu, F. W., Guo, F., Nielsen, E., 2011. A role for CSLD3 during cell-wall synthesis in apical plasma membranes of tip-growing root-hair cells. *Nat. Cell Biol.* 13, 973-U227.

Sletten, E. M., Bertozzi, C. R., 2009. Bioorthogonal chemistry: fishing for selectivity in a sea of functionality. *Angew. Chem. Int. Ed.* 48, 6974-6998.

Somerville, C., Bauer, S., Brininstool, G., Facette, M., Hamann, T., Milne, J., Osborne, E., Paredes, A., Persson, S., Raab, T., Vorwerk, S., Youngs, H., 2004. Toward a systems approach to understanding plant cell walls. *Science* 306, 2206-2211.

Stone, B. A., Evans, N. A., Bonig, I., Clarke, A. E., 1984. The application of Sirofluor, a chemically defined fluorochrome from aniline blue for the histochemical detection of callose. *Protoplasma* 122, 191-195.

Sutherland, E. W., Cohn, M., Posternak, T., Cori, C. F., 1949. The mechanism of the phosphoglucomutase reaction. *J. Biol. Chem.* 180, 1285-1295.

Thevenaz, P., Unser, M., 2007. User-friendly semiautomated assembly of accurate image mosaics in microscopy. *Microsc. Res. Tech.* 70, 135-146.

Thiele, K., Wanner, G., Kindzierski, V., Jurgens, G., Mayer, U., Pahl, F., Assaad, F. F., 2009. The timely deposition of callose is essential for cytokinesis in Arabidopsis. *Plant J.* 58, 13-26.

Vissenberg, K., Fry, S. C., Verbelen, J. P., 2001. Root hair initiation is coupled to a highly localized increase of xyloglucan endotransglycosylase action in Arabidopsis roots. *Plant Physiol.* 127, 1125-1135.

Worden, N., Park, E., Drakakaki, G., 2012. Trans-Golgi network-an intersection of trafficking cell wall components. *J. Integr. Plant Biol.* 54, 875-886.

Figures

Figure 1

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