

1 **Title: Brassinosteroids Influence Arabidopsis Hypocotyl Gravidresponses Through Changes In**  
2 **Mannans And Cellulose**

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4 Running head: Shoot gravidresponses rely on wall polymer changes

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46

47 **Abstract**

48

49 The force of gravity is a constant environmental factor. Plant shoots respond to gravity through  
50 negative gravitropism and gravity resistance. These responses are essential for plants to direct the  
51 growth of aerial organs away from the soil surface after germination and to keep an upright posture  
52 above ground. We took advantage of the effect of brassinosteroids on the two types of graviresponses  
53 in *Arabidopsis thaliana* hypocotyls to disentangle functions of cell wall polymers during etiolated shoot  
54 growth. The ability of etiolated *Arabidopsis* seedlings to grow upwards was suppressed in the  
55 presence of 24-epibrassinolide (EBL) but enhanced in the presence of brassinazole (BRZ), an inhibitor  
56 of brassinosteroid biosynthesis. These effects were accompanied by changes in cell wall mechanics  
57 and composition. Cell wall biochemical analyses and confocal microscopy of the cellulose-specific  
58 pontamine S4B dye revealed that the EBL and BRZ treatments correlated with changes in cellulose  
59 fibre organization and mannan content. Indeed, a longitudinal re-orientation of cellulose fibres  
60 supported upright growth whereas the presence of mannans reduced gravitropic bending. The  
61 negative effect of mannans on gravitropism is a new function for this class of hemicelluloses,  
62 highlighting evolutionary adaptations by which aquatic ancestors of terrestrial plants colonized land.

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64 **Key words:** *Arabidopsis thaliana*, brassinosteroids, cellulose, cell wall, gravitropism, mannan

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## 68 Introduction

69

70 All growing plant cells are encased in primary cell walls, which are strong to maintain cell and tissue  
71 integrity, yet extensible to allow for growth (Cosgrove 2016). How these conflicting properties are  
72 achieved is uncertain because the exact architecture of primary cell walls is not well defined  
73 (Cosgrove 2018). The traditional cell wall model, in which a load-bearing network of cellulose  
74 microfibrils, cross-linked by hemicelluloses, is embedded into an amorphous matrix of pectins and  
75 structural glycoproteins (Carpita and Gibeaut 1993), has been substantially modified and improved  
76 (Cavalier et al. 2008; Dick-Perez et al. 2011). These modified models include a wall load-bearing  
77 capacity that is defined by either lateral cellulose/xyloglucan/cellulose contacts in restricted areas  
78 called “biomechanical hotspots” (Park and Cosgrove 2012), and/or cellulose/pectin interactions (Phyo  
79 et al. 2017a; Phyo et al. 2017b), and/or arabinogalactan proteins covalently linked with pectin and  
80 arabinoxylans (Tan et al. 2013). Additionally, cellulose microfibrils are packed into macrofibrils in  
81 primary cell walls (Anderson et al. 2010), the effect of which on the wall properties is yet to be  
82 determined. At present, there is little consensus on the role of particular polysaccharides in cell walls,  
83 and there is therefore a need for experimental models that could help understand their functions and  
84 interactions.

85 Plant shoots normally grow upright, against the gravity vector, which is supported by two  
86 principal graviresponses – gravitropism and gravity resistance. Gravitropism is defined as directed  
87 growth of a plant or plant organ in response to gravity (Kiss 2000). Negative shoot gravitropism takes  
88 a form of upward bending, an asymmetric shoot growth that restores its vertical position after  
89 inclination (Morita 2010). This response requires flexibility of cell wall polymers and is affected by their  
90 compression resistance on the concave side of the gravitropically bending organ. As such,  
91 gravitropism is a useful model for studying these rarely addressed properties of wall polymers. Shoot  
92 gravity resistance is defined as mechanical resistance to the gravitational force (Hoson and  
93 Wakabayashi 2015). During upright shoot growth, supported by gravity resistance, the conflicting  
94 properties of growing walls are especially prominent: their extensibility should be delicately balanced  
95 with the strength not only to maintain cell and tissue integrity, but also to carry the organ’s weight in  
96 the field of gravity. The fine balance of the opposite wall properties in this system implies that even  
97 minor experimentally imposed modifications of cell wall polymers will be translated into changes of  
98 shoot posture. Hence, the upright shoot growth may work as a sensitive model for establishing the  
99 structural basis of conflicting wall properties.

100 Brassinosteroids (BRs) constitute a class of phytohormones (Singh and Savaldi-Goldstein  
101 2015) that impact graviresponses of *Arabidopsis* hypocotyls that are largely built of primary cell walls  
102 (Vandenbussche et al. 2011). The gravity resistance of hypocotyls was suppressed by epibrassinolide  
103 (EBL), one of the most active natural BRs, while brassinazole (BRZ), a specific inhibitor of BR  
104 biosynthesis, stimulated their gravitropism. These effects did not result from modified gravity  
105 perception but were accompanied by changes in cell wall mechanics (Vandenbussche et al. 2011).  
106 The biochemical basis of the BR-induced alterations in wall physical properties is, however, largely  
107 unknown. Comprehensive microarray data on BR effects on *Arabidopsis* plants revealed changes in

108 many dozens of cell wall-related genes (Goda et al. 2002; Song et al. 2009; Sun et al. 2010; Yu et al.  
109 2011). Thus, the action of BRs, including that of EBL and BRZ, on Arabidopsis hypocotyl  
110 graviresponses could be mediated by a number of modifications at the cell wall level.

111 In the present work we used the strong EBL and BRZ effects on Arabidopsis hypocotyl  
112 graviresponses to better understand cell wall polysaccharide functions employing high-resolution  
113 confocal microscopy, biochemical and biomechanical analyses.

114

115

## 116 **Results**

117

### 118 *Brassinosteroids strongly affect etiolated shoot graviresponses in Arabidopsis*

119 To corroborate that the graviresponses of etiolated Arabidopsis seedlings were altered upon changes  
120 in BR signaling (Vandenbussche et al. 2011), we grew seedlings on horizontal Petri plates in the  
121 presence of exogenous EBL or BRZ and counted upright hypocotyls (Fig. 1). Plants grown on one-half  
122 strength MS media had 40-80% of upright hypocotyls (Fig. 1A). Media supplemented with EBL (100  
123 nM) resulted in a decreased proportion of standing hypocotyls to 0-5% (Fig. 1B), while media  
124 containing BRZ (1  $\mu$ M) increased it to 90-100% (Fig. 1C). These observations were consistent  
125 essentially from the moment of germination and demonstrated that BRs suppressed normal shoot  
126 graviresponses.

127

### 128 *Brassinosteroids influence etiolated shoot graviresponses via changes in cell wall mechanics*

129 The influence of EBL on the hypocotyl posture (Fig. 1B) was hypothesized to emanate from cell wall  
130 weakening that resulted in a loss of hypocotyl gravity resistance and their inability to stand upright in  
131 the field of gravity (Vandenbussche et al. 2011). EBL had no effect on gravitropic bending in  
132 Arabidopsis plants grown on vertical Petri plates and gravistimulated by a 90 degrees clockwise  
133 rotation of the plates (see Fig. 4 in Vandenbussche et al. 2011). In contrast, BRZ increased both the  
134 percentage of upright hypocotyls and their gravitropism, but its effect on cell wall mechanics remained  
135 unclear (Vandenbussche et al. 2011).

136 We used the creep (constant-load) method to assess the shoot gravity resistance. Whenever  
137 hypocotyl length afforded, we stretched their basal regions, carrying the main part of the organ weight  
138 in the field of gravity, as well as their apical growing regions that are responsible for gravitropic  
139 bending (Fig. 2A). The scheme in Fig. 2A illustrates the established age-dependent shift of growth  
140 zones in etiolated Arabidopsis hypocotyls; from the base towards cotyledons (Gendreau et al. 1997;  
141 Bastien et al. 2016). As BRZ greatly inhibited etiolated hypocotyl elongation (Fig. 1A, C), we compared  
142 cell wall creep in BRZ-grown plants with their age-matched 6-day-old untreated counterparts, as well  
143 as with 3-day-old untreated "genetic controls", which are approximately the same length as the 6-day-  
144 old BRZ-grown plants (Fig. 2B). BRZ significantly decreased creep rates compared with both controls,  
145 which is consistent with cell wall strengthening. Thus, the inhibition of BR biosynthesis may increase  
146 the percentage of upright hypocotyls, not only through the stimulation of gravitropic bending (Fig. 4 in  
147 Vandenbussche et al. 2011), but also by increasing their gravity resistance (Fig. 2B). The creep rate

148 analyses in EBL-grown plants revealed cell wall weakening that was mostly restricted to the basal  
149 nonexpanding hypocotyl zones (Fig. 2C-E). Hence, EBL may affect the posture of etiolated hypocotyls  
150 (Fig. 1B) by decreasing their gravity resistance in the basal regions (Fig. 2D-E) where cell expansion  
151 has already ceased (Gendreau et al. 1997).

152

### 153 *Changes in cell wall biochemistry underpin the effects of BRs on graviresponses*

154 To investigate how the cell walls were altered during BR exposure, thereby influencing cell wall  
155 mechanics and the hypocotyl posture, we performed standard cell wall biochemical analyses (Fig. 3).  
156 The content of uronic acids, which are the principal constituents of pectins, was unaffected by the EBL  
157 or BRZ treatments (Fig. 3A). By contrast, crystalline cellulose levels were significantly decreased in  
158 BRZ treated seedlings as compared with the untreated control, while EBL had no effect on this  
159 polymer level (Fig. 3B). As for monosaccharide composition of cell wall matrix polymers, both EBL and  
160 BRZ treatments significantly increased rhamnose content (Fig. 3C). In addition, BRZ treatment  
161 decreased mannose and increased glucose in cell wall matrix polysaccharides (Fig. 3C). Changes in  
162 rhamnose are likely due to the metabolism of rhamnogalacturonan I, which is the main source of this  
163 monosaccharide in primary cell walls. As EBL and BRZ caused opposite effects on the hypocotyl  
164 posture (Fig. 1B, C) but induced very similar increases in rhamnose (Fig. 3C), we argued that it is  
165 unlikely that this sugar is underpinning our observed phenotype. We did therefore not consider the  
166 rhamnose change in further details. Mannose is the principal constituent of cell wall mannans and the  
167 BRZ-induced decrease of mannose could therefore be caused by partial mannan depletion in the  
168 seedling cell walls. The BRZ-induced increase in glucose was not accompanied by any xylose  
169 accumulation (Fig. 3C) indicating that it was not associated with xyloglucans. On the other hand, the  
170 increase in glucose correlated with a decrease in crystalline cellulose (Fig. 3B). The inverse  
171 relationship between crystalline cellulose (Fig. 3B) and glucose (Fig. 3C) could be explained by a  
172 larger amorphous component of cellulose microfibrils, which is likely to be sensitive to TFA hydrolysis,  
173 perhaps explaining the apparent increase in glucose content in BRZ treated seedlings (Fig. 3C).

174

### 175 *EBL effect on the posture of etiolated hypocotyls is related to cellulose arrangement*

176 Rather unexpectedly, the strong effect of EBL on cell wall mechanics (Fig. 2C-E) was not associated  
177 with prominent changes in cell wall biochemical composition (Fig. 3). Nevertheless, not only the levels  
178 of certain cell wall polymers, but also their orientations and interactions in the wall affect cell wall  
179 mechanics, with implications both on cellular strength and expansion. This is especially true for  
180 cellulose, the strongest cell wall component (Suslov and Verbelen 2006; Suslov et al. 2009). To  
181 examine the orientation of cellulose in outer epidermal cell walls of hypocotyls from control and EBL-  
182 grown plants we used the specific fluorescent dye Pontamine Fast Scarlet 4B (S4B) (Anderson et al.  
183 2010). We imaged the dye-associated cellulose fibers using spinning-disc or high-resolution Airyscan  
184 confocal microscopy. With these setups we could reveal distinct cellulose macrofibril orientations. To  
185 improve the penetration of the dye and visualization of the cellulose fibers along the whole hypocotyl  
186 length we performed a mild extraction of cell walls. Without this treatment the cellulose fibers were not  
187 well discerned in the basal parts of living hypocotyls. Nevertheless, living and extracted seedlings

188 displayed similar cellulose arrangements in the upper growing region of their hypocotyls. In this part of  
189 control seedlings transverse buckling was frequently observed in the innermost wall layer. This  
190 phenomenon occurred in approximately 50% of both living (Supplementary Fig. S1) and extracted  
191 control hypocotyls, and complicated cellulose macrofibril visualization on the inner wall face. In the  
192 remaining samples, i.e. in which we did not observe the buckling, cellulose macrofibrils were clearly  
193 transverse in the innermost wall layer and longitudinal in the outermost layer (Supplementary Video  
194 S1; Fig. 4A; Table 1). In the basal non-growing region of control hypocotyls, we observed slight or no  
195 buckling of the wall inner surface. These walls contained less transverse macrofibrils in the innermost  
196 layer and thicker longitudinal macrofibrils in the outermost layer compared with the upper growing  
197 region of hypocotyls (Supplementary Video S2; Fig. 4D; Table 1). In the upper region of approximately  
198 50% of hypocotyls from EBL-grown plants, we observed irregular buckling in the innermost wall. In the  
199 samples without buckling, cellulose macrofibrils were more obliquely oriented (Fig. 4B), or decreased  
200 in relative abundance, compared with the untreated control. EBL did not influence longitudinal  
201 macrofibrils in the outermost wall layer in the growing zone of hypocotyls (Supplementary Video S3).  
202 The most dramatic EBL effect on cellulose orientation was found in the basal region of hypocotyls  
203 where it essentially eliminated longitudinal macrofibrils, such that the remaining ones were apparently  
204 thinner and had either oblique or random orientation (Fig. 4E; Supplementary Video S4; Table 1). This  
205 reduction in longitudinal macrofibrils at the hypocotyl base was a unique effect observed only in EBL-  
206 treated seedlings (Table 1; Fig. 4).

207 To confirm that the EBL effect on hypocotyl posture was associated with reduced macrofibril  
208 organization, we attempted to “randomize” cellulose orientation in the cell walls and then see how this  
209 affected the percentage of standing hypocotyls (Fig. 5). For this purpose, Arabidopsis seedlings were  
210 grown in the presence of 250 nM oryzalin, which partially disassembles cortical microtubules, thereby  
211 affecting the direction of cellulose microfibril deposition in the cell wall (Paredes et al. 2006). This low  
212 concentration of oryzalin induced similar changes in cellulose arrangement as those observed with  
213 EBL in the upper part of hypocotyls (compare Supplementary Video S5 with Supplementary Video S3;  
214 and Fig. 4B with 4G; Table 1), and significantly decreased the percentage of standing hypocotyls (Fig.  
215 5). Thus, intact cellulose orientation is important for keeping the hypocotyls upright, and the  
216 mechanism of EBL action on their posture may be based on changes in cellulose arrangement.  
217 However, the effect of oryzalin on the percentage of upright hypocotyls (Fig. 5) was weaker than that  
218 of EBL (Fig. 1B). This difference can be explained by the inability of oryzalin to alter longitudinal  
219 cellulose macrofibrils in the basal region of hypocotyls, in contrast to the effect of EBL (compare  
220 Supplementary Video S6 with Supplementary Video S4; and Fig. 4E with Fig. 4I; Table 1). These  
221 longitudinal macrofibrils accumulated in the outermost wall layer where they could contribute to the  
222 mechanical strength and upright growth of hypocotyls. Their reduced presence in EBL-treated  
223 seedlings (Fig. 4E, Supplementary Video S4) correlated with weaker walls at the base of hypocotyls  
224 (Fig. 2D, E).

225

226 *BRZ increases the percentage of upright etiolated hypocotyls via several different mechanisms*

227 Interestingly, BRZ increased the percentage of upright hypocotyls independently of oryzalin treatment  
228 (250 nM) (Fig. 5). These data indicate that BRZ either regulates graviresponses somewhat  
229 independently of cellulose orientation, and/or antagonizes the oryzalin-induced microfibril  
230 randomization. Our microscopic observations supported the first option, because we observed less  
231 ordered cellulose orientations in the upper parts of seedlings treated with oryzalin and BRZ (Fig. 4C,  
232 H; Supplementary Videos S7, S8; Table 1). At the same time the cellulose arrangement in the basal  
233 hypocotyl part was essentially similar in the untreated controls and seedlings grown in the presence of  
234 BRZ, oryzalin, and BRZ plus oryzalin (Fig. 4D, F, I, J; Supplementary Videos S2, S6, S9, S10; Table  
235 1).

236 Thus, our imaging data indicate that BRZ may affect graviresponses via mechanisms not  
237 related to cellulose. One such mechanism might be mediated by reducing the mannan content (Fig.  
238 3C). To assess whether a decrease in mannan content affected the graviresponses we studied triple  
239 *cs1a2cs1a3cs1a9* mutant plants lacking detectable glucomannans in mature Arabidopsis stems (Goubet  
240 et al. 2009). Transcriptomics data indicate that out of the nine *CSLA* genes in Arabidopsis, *CSLA2*, 3,  
241 and 9 have the highest expression in hypocotyls (Winter et al. 2007). The *cs1a2cs1a3cs1a9* triple  
242 mutation did not influence the percentage of standing hypocotyls (Supplementary Fig. S2). However,  
243 by analogy with BRZ, the mutants displayed significantly accelerated gravitropic bending in  
244 reorientation assays with plants grown on vertical Petri plates (Fig. 6; Supplementary Fig. S3).  
245 Interestingly, the coefficient of BRZ stimulation of gravitropic bending was reduced in the background  
246 of *cs1a2cs1a3cs1a9* compared with Col-0 (Fig. 6, Table 2). The above findings show that the decreased  
247 mannan content (Fig. 3C) accounts – at least in part - for the BRZ-induced increase in gravitropic  
248 bending.

249 Plant cell expansion is known to be induced by cell wall loosening (Cosgrove 2016; Ivakov et  
250 al. 2017). Cell growth is slow and uniform along the length of young etiolated hypocotyls that have just  
251 emerged from germinating seeds (Refrégier et al. 2004). A wave of rapid cell expansion starts at the  
252 base of hypocotyls at about 48 h post induction, after which it spreads acropetally towards cotyledons  
253 (Gendreau et al. 1997; Bastien et al. 2016). Hence, cell wall loosening is highly induced in the basal  
254 region of hypocotyls carrying their main weight, which can interfere with keeping the upright position of  
255 this organ in the field of gravity. As the effect of BRZ on hypocotyl posture is visible from the moment  
256 of emergence from the seed, it may be caused by changes in cell expansion and, hence, cell wall  
257 loosening in very young hypocotyls. To test this option, cell length was measured in epidermal cell  
258 files at two developmental stages (Fig. 7A), allowing growth rate calculation for individual cells along  
259 hypocotyls (Fig. 7B). Only basal halves of epidermal cell files along hypocotyls (ten lower cells) were  
260 considered. These are the regions where the rapid growth is first initiated in hypocotyls (Gendreau et  
261 al. 1997). Cell length was measured at 55 h post induction, the time point corresponding to the earliest  
262 phase of rapid growth in hypocotyls (Pelletier et al. 2010), and at 72 h post induction, when maximal  
263 growth rate is attained (Gendreau et al. 1997). Epidermal cell length was significantly lower in  
264 hypocotyls of BRZ-treated vs. control plants for all cells with the exception of the first cell at 55 h  
265 illustrating severe growth inhibition due to a decrease in BR biosynthesis (Fig. 7A). The rate of cell  
266 expansion was also significantly lower for the majority of cells in BRZ-treated vs. control plants



267 indicating that the wall loosening was inhibited in the former (Fig. 7B). Interestingly, two peaks of  
268 growth inhibition were observed in BRZ-treated seedlings: at the base of hypocotyls (cells one and  
269 three) and in their most apical zone examined (cells nine and ten) (Fig. 7B). Thus, the wave of rapid  
270 growth is initiated in a higher region of hypocotyls and spreads acropetally slower in BRZ-treated vs.  
271 control plants. Overall, the above data indicate that cell wall loosening is inhibited more strongly at the  
272 base of hypocotyls, i.e. in the region responsible for carrying the seedling weight in the field of gravity.  
273 This inhibition can be another mechanism by which BRZ increases the percentage of standing  
274 hypocotyls.

275

276

## 277 **Discussion**

278

279 The two prerequisites for upright growth of young shoots, mechanical strength and gravitropic  
280 bending, are based on distinct physiological mechanisms, which are linked through the cell wall  
281 characteristics. The mechanical strength of young shoots is defined by turgor (Shah et al. 2017). This  
282 is well illustrated by wilting, a loss of turgor, upon which young shoots fall down on the ground. Turgor  
283 depends on the transmembrane concentration gradient of osmotically active compounds, the hydraulic  
284 conductance and wall yielding properties (Ray et al. 1972). The equation for turgor (Ray et al. 1972)  
285 implies that cell wall weakening (Fig. 2C-E) and strengthening (Fig. 2B) in the presence of EBL and  
286 BRZ, respectively, will contribute to lower and higher turgor values, which could underpin the BR  
287 effects on hypocotyl posture (Fig. 1). However, we cannot exclude that these effects are partially  
288 mediated by osmotic adjustments also influencing turgor pressure, which was not addressed here.  
289 Similarly, the selective BRZ-induced growth inhibition at the hypocotyl base (Fig. 7B), presumably  
290 mediated by decreased cell wall yielding, could elevate turgor, thereby maintaining their upright  
291 position.

292 Gravitropic bending results from asymmetric cell expansion on the opposite flanks of  
293 gravistimulated organs (Miller et al. 2007). In young shoots put horizontally this response originates  
294 from simultaneous growth inhibition and stimulation on their upper and lower flanks, respectively  
295 (Bagshaw and Cleland 1990; Cosgrove 1990a; Ikushima et al. 2008). As in the case of cell expansion  
296 in vertical plant organs (Cosgrove 2018), the growth responses during gravitropic bending are  
297 controlled by cell wall yielding properties (Bagshaw and Cleland, 1990; Cosgrove 1990b; Edelmann  
298 and Samajova 1999; Ikushima et al. 2008). Cell wall extensibility always decreases on the upper sides  
299 and sometimes increases on the lower sides of gravistimulated shoots, such that the overall cell wall  
300 loosening or tightening prevails depending on the species (Edelmann 1997) and/or the phase of  
301 gravitropic bending (Bagshaw and Cleland 1990). However, this prevalent overall loosening and  
302 tightening will not affect the percentage of standing 5- and 6-day old Arabidopsis hypocotyls in which  
303 the zone of gravitropic bending is physically separated from the basal zone that is mostly responsible  
304 for the mechanical strength needed for their upright posture (Fig. 2A).

305 Cellulose is the strongest cell wall component. The requirement of high wall mechanical  
306 strength for keeping the shoot upright indicates that the arrangement of microfibrils could affect this

307 process. The modulus of individual cellulose microfibrils ranges from 0.7 to 3.5 GPa (Chanliaud et al.  
308 2002), which is about 100-fold greater than the tensile modulus of primary cell walls (Ryden et al.  
309 2003). Due to this fact, cellulose microfibrils never extend axially under physiological conditions,  
310 instead they separate from each other, bend, slide past each other and reorient in the direction of  
311 strain during growth, or under external forces, resulting in cell wall deformations (Preston 1982;  
312 Refrégier et al. 2004; Zhang et al. 2017). The cellulose mobility depends on microfibril-microfibril and  
313 microfibril-matrix interactions, proteinaceous cell wall loosening/tightening factors (Chanliaud et al.  
314 2002; Chanliaud et al. 2004; Zhang et al. 2017) and cellulose orientation that is defined by cortical  
315 microtubules (Bringmann et al. 2012; Paredez et al. 2006). BRs affect many processes linked with  
316 cellulose. They transcriptionally regulate the majority of *CESA* genes encoding catalytic subunits of  
317 cellulose synthase complexes through the involvement of the BR-activated transcription factor BES1  
318 (Xie et al. 2011). Additionally, BRs influence cellulose synthesis post-translationally: the negative  
319 regulator of BR signaling, BRASSINOSTEROID INSENSITIVE2 (BIN2), can phosphorylate one of the  
320 cellulose synthase subunits that leads to impaired cellulose synthesis (Sanchez-Rodriguez et al.  
321 2017). These mechanisms can be the cause for the BRZ-induced decrease in cellulose content (Fig.  
322 3B). BRs may also change microfibril orientation as they influence cortical microtubule organization  
323 (Gupta et al. 2012), possibly via BIN2 (Liu et al. 2018). Hence, the EBL-induced formation of oblique  
324 cellulose macrofibrils in the inner wall layer adjacent to the plasma membrane (Fig. 4B, E; Table 1;  
325 Supplementary Videos S3, S4) may result from cortical microtubule reorientations.

326 We observed two additional cellulose-related effects of BRs. First, the BRZ-induced decrease  
327 in crystalline cellulose and increase in the TFA-released glucose, without increase in xylose (Fig. 3B,  
328 C), can be interpreted as a decrease in the cellulose crystallinity. Similar observations were made in  
329 *isoxaben resistant (ixr)* mutants (DeBolt et al. 2013). BRZ could thus generate cellulose microfibrils  
330 with reduced crystallinity and more exposed glucan chains. This might provide a larger surface area,  
331 and thus additional sites, for noncovalent interactions with adjacent microfibrils and matrix  
332 polysaccharides. The 'rough' surface of such microfibrils could also provide a better access for  
333 enzymes forming covalent cross-links between cell wall components. A good candidate for this role is  
334 AtXTH3 that catalyses cellulose-cellulose, cellulose-xyloglucan and xyloglucan-xyloglucan  
335 transglycosylation (Shinohara et al. 2017). All these additional noncovalent and covalent interactions  
336 could explain the increased wall mechanical strength observed with BRZ treatment (Fig. 2B) that  
337 contributes to the upright posture of hypocotyls (Fig. 1C).

338 The second cellulose-related effect of BRs is the EBL-induced macrofibril disorganization at  
339 the base of hypocotyls (Fig. 4E; Supplementary Video S4). The overall arrangement of macrofibrils  
340 along *Arabidopsis* hypocotyls is consistent with the classical multinet growth theory, according to  
341 which cellulose is deposited transversely next to the plasma membrane, followed by a passive  
342 microfibril displacement into deeper wall layers and their axial reorientation, i.e. parallel to the direction  
343 of maximal growth (Preston 1982; Refrégier et al. 2004; Roelofsen 1958). As epidermal cells at the  
344 base of hypocotyls are at a later developmental stage than those at the apex (Bastien et al. 2016;  
345 Gendreau et al. 1997), it means that the majority of longitudinal macrofibrils in the outer wall layer are  
346 post-synthetically re-oriented to produce oblique ones in fully elongated cells. A force that drives such

347 re-orientation must be very high. The most likely candidate for this role is a change in the wall  
348 hydration generating forces that can rearrange cellulose microfibrils (Huang et al. 2018). In line with  
349 this hypothesis, BRs were shown to induce a rapid wall swelling (Caesar et al. 2011). Irrespectively of  
350 the mechanisms involved, the elimination and thinning of longitudinal cellulose macrofibrils observed  
351 at the base of hypocotyls from EBL-grown plants could decrease their resistance to bending, such that  
352 they can easily curve under their own weight and fall on the agar surface (Fig. 1B), which would  
353 decrease the percentage of standing hypocotyls. It is, however, important to note that the EBL and  
354 BRZ treatment may also affect the content and distribution of other hormones that may contribute to  
355 the observed effects.

356 Mannans are minor hemicelluloses of primary cell walls in angiosperms. Their backbones are  
357 either composed of (1→4)-β-D-mannose residues only (pure mannan), or contain (1→4)-β-linked D-  
358 glucose and D-mannose residues distributed along the chain in a non-regular fashion (glucomannan).  
359 Both polysaccharides can have (1→6)-α-D-galactose substitutions on their backbones, in which case  
360 they are referred to as galactomannans and galactoglucomannans, respectively (Schröder et al.  
361 2009). Mannans are considered to play a structural role in cell walls based on experiments with cell  
362 wall analogues (Whitney et al. 1998). However, doubts have been cast on the structural functions of  
363 mannans in cell walls based on the phenotype of Arabidopsis triple mutant *cs/a2cs/a3cs/a9*. Being  
364 deficient in mannan biosynthesis and containing no detectable glucomannans, the mutant plants were  
365 phenotypically indistinguishable from their wild type counterparts (Goubet et al. 2009). Our findings  
366 that BRZ decreases the content of mannose in cell wall matrix polysaccharides (Fig. 3C), while  
367 increasing the gravitropic bending of hypocotyls (Vandenbussche et al. 2011; Fig. 6), and that the  
368 triple mutation *cs/a2cs/a3cs/a9* increases the gravitropic curvature (Fig. 6) with no effect on the  
369 percentage of upright hypocotyls (Supplementary Fig. S2) show that mannans have a negative effect  
370 on gravitropic bending. This role in shoot gravitropism is a new function for mannan polysaccharides.  
371 Interestingly, our data are consistent with the fact that gravistimulation of wild type Arabidopsis  
372 seedlings was accompanied by a strong downregulation of one gene (*ATCSLA15*) responsible for  
373 mannan biosynthesis (Millar and Kiss 2013). Additionally, the level of galactoglucomannans was  
374 decreased by about 50% in maize stem pulvini as a result of gravistimulation (Zhang et al. 2011). The  
375 negative influence of mannans on shoot gravitropism is interesting from an evolutionary perspective.  
376 Aquatic ancestors of terrestrial plants used buoyancy to support their bodies (Hejnowicz 1997) and  
377 contained mannans and xylans as principal hemicelluloses (Popper and Tuohy 2010). Interestingly,  
378 xyloglucans, a class of hemicelluloses that became prevalent in the primary cell walls of many  
379 terrestrial plants, emerged in charophycean green algae (Sørensen et al. 2010), which are considered  
380 land plant ancestors (Wickett et al. 2014). It thus appears that xyloglucans are better suited than  
381 mannans for land habitats, which could relate to their involvement in plant gravitropism (Velasquez et  
382 al. 2019). In this evolutionary context it is important to find out why mannans interfere with gravitropic  
383 bending. The precise mechanism of the effect is unknown, but we consider three hypotheses:

384 1) Glucomannans have less regularly organized backbones than xyloglucans (Schröder et al.  
385 2009) making them insufficiently flexible alone or in a complex with different cell wall components to  
386 support the rapid formation of gravitropic bending.

387 2) Gravitropic bending may involve cellulose reorientations because microtubules, the key  
388 determinants of cellulose alignment (Paredes et al. 2006), demonstrated very similar reorientations  
389 during gravitropism in different plant organs and species (Blancaflor and Hasenstein 1995;  
390 Himmelspach et al. 1999; Zhang et al. 2008). At the same time, mannans were shown to affect  
391 cellulose organisation (Yu et al. 2014). Hence, these hemicellulosic polymers may also change  
392 cellulose reorientations in the course of gravitropism, which could influence its rate.

393 3) Changes in mannan synthesis can affect gravitropic bending by modulating the level of  
394 reactive oxygen species (ROS). This scenario is possible because GDP-mannose is a common  
395 precursor in the biosynthesis of mannans and ascorbate (Sawake et al. 2015). Accordingly, the  
396 downregulation of *CSLA* gene expression by gravistimulation (Millar and Kiss 2013) may increase the  
397 GDP-mannose pool available for ascorbate biosynthesis. The resulting increase in ascorbate, a strong  
398 antioxidant, could modulate ROS levels, which are important for gravitropic responses (Joo et al.  
399 2001; Krieger et al. 2016; Singh et al. 2017; Zhou et al. 2018).

400 These mechanisms are not mutually exclusive. Studying their contribution to shoot  
401 gravitropism will shed light to the role of mannans in cell wall architecture. This could also improve our  
402 understanding of key cell wall modifications that allowed successful land colonization by aquatic  
403 ancestors of terrestrial plants.

404

405

## 406 **Materials and Methods**

407

### 408 *Plant material and growth conditions*

409 *Arabidopsis thaliana* (L. Heynh.) wild type Columbia-0 and mutant plants were grown on half-strength  
410 Murashige and Skoog (MS) medium (pH 5.7) (Duchefa Biochemie) containing 0.68% (w/v) microagar  
411 (Duchefa Biochemie). Where indicated, the medium was supplemented with stock solutions of  
412 epibrassinolide (0.2 mM in methanol), brassinazole (2 mM in methanol) and oryzalin (500  $\mu$ M in  
413 ethanol), such that their final concentrations were 100 nM, 1  $\mu$ M and 250 nM, respectively. In each  
414 case the medium for control untreated plants was supplemented with corresponding volumes of  
415 methanol and/or ethanol.

416 Surface-sterilized seeds were sown aseptically on large (145×20 mm) round Petri plates  
417 (Greiner) containing the above-mentioned media. The seeds were stratified for 2 days at 4°C, and  
418 their synchronous germination was induced by exposure to fluorescent white light (150  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>)  
419 for 6 h at 21°C. The moment of transfer to light, i.e. the beginning of induction, was taken as zero age  
420 for experimental plants. After the 6 h induction period the Petri plates were wrapped in two layers of  
421 thick aluminium foil and placed horizontally (or, where indicated, vertically) in an environmentally  
422 controlled growth room. Five-day-old etiolated plants grown at 21°C were used in experiments, unless  
423 specified otherwise.

424

### 425 *Extensometry*

426 Arabidopsis seedlings for creep tests were placed individually into 1.5 ml Eppendorf test tubes, frozen  
427 by plunging the closed tubes into liquid nitrogen, stored at -20°C and used for extensometry within two  
428 weeks after freezing. *In vitro* extension of frozen/thawed hypocotyls was measured with a custom-built  
429 constant load extensometer (Suslov and Verbelen 2006). A 2- or 5-mm-long segment from the  
430 specified region of a hypocotyl was secured between clamps of the extensometer and preincubated in  
431 a buffer (20 mM MES-KOH, pH 6.0) in the relaxed state for 2 min. Then, its time-dependent extension  
432 (creep) was measured in the same buffer under 625 mg or 750 mg loads for 15 min. The relative  
433 creep rate was calculated as described in Vandebussche et al. (2011).

434

#### 435 *Cell wall biochemical analyses*

436 The biochemical analyses of cell wall polysaccharides were performed largely as previously published  
437 (Foster et al. 2010). Approximately 300 of 5-day-old seedlings grown on one Petri plate were  
438 considered as a biological replicate. Each experiment on the wall biochemistry included three  
439 biological replicates, and a total of three independent experiments were performed (i.e. n=9). The  
440 seedlings were rapidly harvested to a large volume of 70% EtOH, after which their seed coats were  
441 manually removed and discarded. The resulting plant material was transferred to a 2 ml Eppendorf  
442 test tube, 1.5 ml of 70% EtOH was added, followed by centrifugation at 10,000 rpm for 5 min  
443 discarding the supernatant. One ml of acetone was added to the resulting pellet, followed by  
444 centrifugation at 10,000 rpm for 5 min. The supernatant was discarded, and the residue was air-dried  
445 overnight. The resulting dry material was ball-milled (Retsch) for 1 min. One ml of EtOH was added to  
446 the residue, followed by centrifugation at 21,000 g for 10 min discarding the supernatant. Then 1 ml of  
447 MeOH:chloroform (1:1) mixture was added, followed by centrifugation at 21,000 g for 10 min,  
448 discarding the supernatant and air-drying the pellet. The resulting powdered cell wall pellet was  
449 weighed and transferred to a clean screw-capped 2 ml plastic Sarstedt test tube. *Myo*-Inositol (30 µl of  
450 1 mg ml<sup>-1</sup> solution in water) was added to the test tube as internal standard. Then 250 µl of 2M TFA  
451 was added, followed by incubation at 121°C for 1 h to hydrolyze cell wall polysaccharides. The tube  
452 was rapidly cooled on ice, 300 µl of 2-propanol was added and evaporated at 40°C, and the step with  
453 2-propanol was repeated two more times. After this 250 µl of H<sub>2</sub>O was added, and the tube was  
454 vortexed, sonicated for 10 min and centrifuged at 21,000 g for 10 min. One part of the resulting  
455 supernatant was taken for uronic acids quantification (2 × 50 µl), and the other portion (100 µl) was  
456 dried and used for assaying neutral cell wall monosaccharides, while the pellet was processed for  
457 crystalline cellulose quantification. Subsequent cell wall biochemical analyses were carried out as  
458 described in Sanchez-Rodriguez et al. (2012). Uronic acids were colorimetrically measured using 2-  
459 hydroxydiphenyl as reagent (Vilím 1985) with galacturonic acid as standard (Filisetti-Cozzi and Carpita  
460 1991). Cell wall monosaccharides were assayed as alditol acetate derivatives (Neumetzler 2010)  
461 using a modified protocol from Albersheim et al. (1967) by gas chromatography performed on an  
462 Agilent 6890N GC system coupled with an Agilent 5973N mass selective detector. Crystalline  
463 cellulose was determined by Seaman hydrolysis (Selvendran and O'Neill 1987) using Glc equivalents  
464 as standard, where the cellulosic Glc content was determined with the anthrone assay (Dische 1962).

465

466 *Microscopy*

467 Cellulose microfibrils were visualized in cell walls with Pontamine Fast Scarlet 4B (S4B) dye  
468 (Anderson et al. 2010). In some experiments, living seedlings were stained in 0.2% (w/v) S4B for 30  
469 min, which revealed microfibrils only in the upper growing parts of hypocotyls. To provide the dye  
470 penetration to the cell walls at the base of hypocotyls, plants were extracted under mild conditions by  
471 sequential washes with EtOH:100% acetic acid (7:1, v/v) for 1 h; 100% EtOH for 15 min; 50% EtOH in  
472 H<sub>2</sub>O for 15 min; and 1M KOH in H<sub>2</sub>O for 30 min. All washing steps were carried out on a rotator.  
473 Experimental samples were stored in 1M KOH at 4°C before analysis or used immediately after the  
474 last alkaline wash for cellulose visualization. The samples were rinsed in H<sub>2</sub>O before staining to  
475 remove residual KOH, after which 0.2% (w/v) S4B was added for 30 min. Then they were rapidly  
476 rinsed with a large volume of H<sub>2</sub>O to remove excessive dye and observed under a spinning disc  
477 confocal microscope or near-super-resolution Airyscan confocal microscope (Zeiss LSM880). The  
478 equipment for spinning disc confocal microscopy included a Nikon Ti-E inverted confocal microscope  
479 equipped with a CSU-X1 spinning disc head (Yokogawa, Japan), a 100x CFI Apo oil immersion TIRF  
480 objective (NA 1.49, Nikon, Japan), an evolve charge-coupled device camera (Photometrics  
481 Technology, USA), and a 1.2x lens between the spinning disc and camera. S4B was excited using a  
482 561 nm laser (similar to Anderson et al. 2010). Image acquisitions were performed using Metamorph  
483 software (Molecular Devices, USA) version 7.5. High-resolution imaging of cellulose microfibrils was  
484 performed on a Zeiss LSM880 microscope equipped with an AiryScan Unit (Huff 2015). S4B was  
485 excited using a 514 nm Argon Laser through a 458/514 MBS and a 63x Plan-Apochromat oil objective  
486 with a numeric aperture of 1.4. The 514 laser was used as 535 nm was indicated as an optimal S4B  
487 excitation wavelength (Anderson et al. 2010), which yielded good images for analyses. Emission was  
488 detected using the 32 GaAsP PMT array AiryScan unit. For each 8-bit image, a Z-stack consisting of  
489 2-5 images with a 1 µm step-size, an image size of 1672x1672 pixels, and with 1.26 µs pixel dwell  
490 time was recorded. The recordings were deconvoluted using the Zeiss ZEN software at highest  
491 possible resolution.

492 Epidermal cell images at the base of hypocotyls were captured with a Leica DM 4000 light  
493 microscope equipped with a 1.3 megapixel CCD camera using a dark field mode, under a HCX PL  
494 FLUOTAR 20x/0.40 CORR objective. Cell lengths were then measured on digital images using a  
495 segmented line tool in ImageJ 1.49b software.

496

497 *Time lapse photography by infrared imaging*

498 For gravitropic reorientation assays, plants were grown in infrared light (930 nm) on vertical plates  
499 containing the half-strength MS medium with microagar. Plates with three-day-old seedlings were  
500 rotated 90° (time point zero). Subsequently, the seedlings were imaged using infrared enabled  
501 cameras (Vandenbussche et al. 2010). Images were analysed using the angle tool in ImageJ.

502

503

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512

513

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515

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517

518

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525

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- 718

719 **Table 1** Overview of cellulose microfibril orientations

720 Cellulose microfibril orientation across the whole thickness of outer epidermal cell walls of  
 721 Arabidopsis hypocotyls was determined using S4B dye, spinning-disc and super-resolution confocal  
 722 microscopy. The prevalence of four types of cellulose microfibril orientation across the wall thickness  
 723 was characterized by scores 1 to 3 as high (3), intermediate (2) or low (1). These values were  
 724 averaged between all cells analyzed for a given treatment and presented as means  $\pm$  SD.

Treatment	Cellulose orientation				Number of repeats <sup>*</sup>
	transverse	oblique	random	longitudinal	
Col-0, upper <sup>†</sup>	1.82 $\pm$ 0.70	0.09 $\pm$ 0.28	0.08 $\pm$ 0.25	1.43 $\pm$ 0.63	38
Col-0, lower <sup>‡</sup>	1.18 $\pm$ 0.56	0.16 $\pm$ 0.37	0.05 $\pm$ 0.27	1.89 $\pm$ 0.51	38
Col-0+EBL, upper	0.42 $\pm$ 0.69	0.47 $\pm$ 0.77	0.10 $\pm$ 0.30	1.53 $\pm$ 0.61	19
Col-0+EBL, lower	0.33 $\pm$ 0.55	1.40 $\pm$ 0.72	0.43 $\pm$ 0.63	0.63 $\pm$ 0.56	30
Col-0+BRZ, upper	1.48 $\pm$ 0.60	0.02 $\pm$ 0.09	0.26 $\pm$ 0.44	1.82 $\pm$ 0.67	31
Col-0+BRZ, lower	0.65 $\pm$ 0.49	0.09 $\pm$ 0.29	0.17 $\pm$ 0.39	1.91 $\pm$ 0.29	23
Col-0+ory, upper	0.90 $\pm$ 0.84	0.52 $\pm$ 0.70	1.22 $\pm$ 0.85	1.56 $\pm$ 0.73	25
Col-0+ory, lower	0.23 $\pm$ 0.44	0.15 $\pm$ 0.38	0.77 $\pm$ 0.60	2.00 $\pm$ 0.00	13
Col-0+ory+BRZ, upper	0.63 $\pm$ 0.66	0.22 $\pm$ 0.36	1.13 $\pm$ 0.67	1.32 $\pm$ 0.75	26
Col-0+ory+BRZ, lower	0.20 $\pm$ 0.41	0.11 $\pm$ 0.32	1.20 $\pm$ 0.70	1.90 $\pm$ 0.31	20

725 <sup>\*</sup>: Each repeat is an outer cell wall from an individual epidermal cell. The data are based on three-four  
 726 independent experiments with at least four seedlings analyzed in each of them. <sup>†</sup>: upper region of a  
 727 hypocotyl. <sup>‡</sup>: lower region of a hypocotyl. EBL: epibrassinolide; BRZ: brassinazole; ory: oryzalin.

728

729 **Table 2** Stimulation of gravitropic bending by brassinazole is impaired in the triple *csla2csla3csla9*  
 730 mutant compared with *Col-0* plants  
 731 Gravitropic bending in the presence of BRZ (Fig. 6) was divided by that without BRZ for *Col-0* and  
 732 *csla2csla3csla9*, respectively.

Comparison	Time after gravistimulation, hours											
	1	2	3	4	5	6	7	8	9	10	11	12
	<i>Stimulation of bending, fold</i>											
<i>Col-0</i> +BRZ vs. <i>Col-0</i>	12.8	13.0	12.4	6.4	3.2	2.5	2.4	1.9	2.0	1.8	1.9	2.0
<i>csla2csla3csla9</i> +BRZ vs. <i>csla2csla3csla9</i>	5.0	4.6	3.2	3.1	2.6	1.8	1.8	1.7	1.7	1.4	1.7	1.5

733

734 **FIGURE LEGENDS**

735 **Fig. 1** Brassinosteroids affect upright growth of hypocotyls in Arabidopsis plants. Etiolated *Col-0*  
736 seedlings were grown on horizontal Petri plates with one-half strength agarized Murashige and Skoog  
737 medium without supplements (A), with 100 nM EBL (B) or 1  $\mu$ M BRZ (C). Five-day-old plants were  
738 photographed.

739 **Fig. 2** Brassinosteroids change cell wall mechanics in Arabidopsis hypocotyls. (A) The schematic  
740 representation of regions extended by the creep method (orange shadings) in hypocotyls of different  
741 age. Arrowheads mark borders of growing zones in hypocotyls of *Col-0* plants of the respective age.  
742 Changes in creep rates by BRZ (1  $\mu$ M) in hypocotyls of 6-day-old *Col-0* plants (B) and by EBL (100  
743 nM) in hypocotyls of 3-day-old (C), 5-day-old (D) and 6-day-old (E) *Col-0* plants. All seedlings in B-E  
744 were grown on horizontal Petri plates. Creep rates were measured under 750 mg (B, C, E) or 625 mg  
745 (D) loads. Data are means  $\pm$ SE (n=10). Different letters in (B) mark significant differences ( $P < 0.05$ )  
746 revealed by Games-Howell's post-hoc test performed after ANOVA. Asterisks in (C-E) denote  
747 significant difference (\* $P < 0.05$ ; \*\* $P < 0.01$ ) between the respective zones of EBL-grown and control  
748 seedlings determined by Student's *t*-test.

749 **Fig. 3** Biochemical composition of cell walls in Arabidopsis plants as affected by brassinosteroids. The  
750 levels of uronic acids (A), crystalline cellulose (B) and the monosaccharide composition of cell wall  
751 matrix polymers (C) were determined in 5-day-old etiolated *Col-0* seedlings grown on horizontal Petri  
752 plates without supplements in the growth medium (control), in the presence of 1  $\mu$ M BRZ or 100 nM  
753 EBL. Data are means  $\pm$ SE (n=9). Asterisks denote significant differences (\* $P < 0.05$ ; \*\* $P < 0.01$ )  
754 revealed by Dunnett's post-hoc test performed after ANOVA.

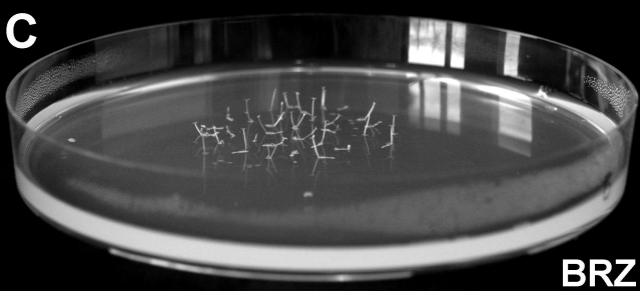
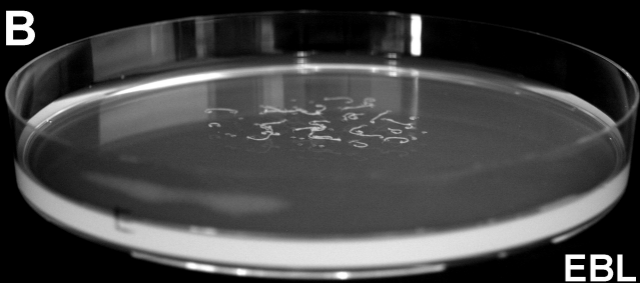
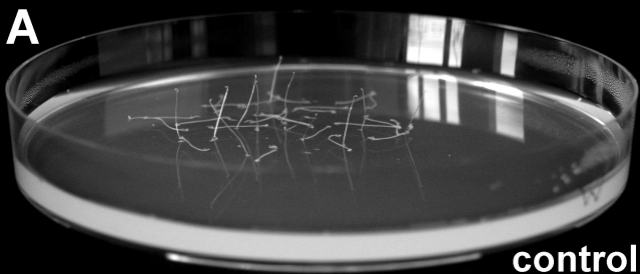
755 **Fig. 4** Brassinosteroid and oryzalin effects on the arrangement of cellulose microfibrils in the outer  
756 epidermal cell wall of hypocotyls from 5-day-old etiolated *Col-0* Arabidopsis plants grown on horizontal  
757 Petri plates. Hypocotyls were extracted under mild conditions, their walls were stained with Pontamine  
758 Fast Scarlet 4B, the cellulose-specific fluorescent dye, and observed under a super-resolution  
759 Airyscan confocal microscope. Cellulose orientation in projections of three-five optical sections across  
760 the whole wall thickness is shown (A) in the upper growing and (D) the lower non-growing region of  
761 hypocotyls from control seedlings grown on one-half strength agarized Murashige and Skoog medium  
762 without supplements; (B) the upper and (E) the lower region of hypocotyls from plants grown in the  
763 presence of 100 nM EBL; (C) the upper and (F) the lower region of hypocotyls from plants grown in  
764 the presence of 1  $\mu$ M BRZ; (G) the upper and (I) the lower region of hypocotyls from plants grown in  
765 the presence of 250 nM oryzalin; (H) the upper and (J) the lower region of hypocotyls from plants  
766 grown in the presence of 1  $\mu$ M BRZ and 250 nM oryzalin. Scale bars are 10  $\mu$ m.

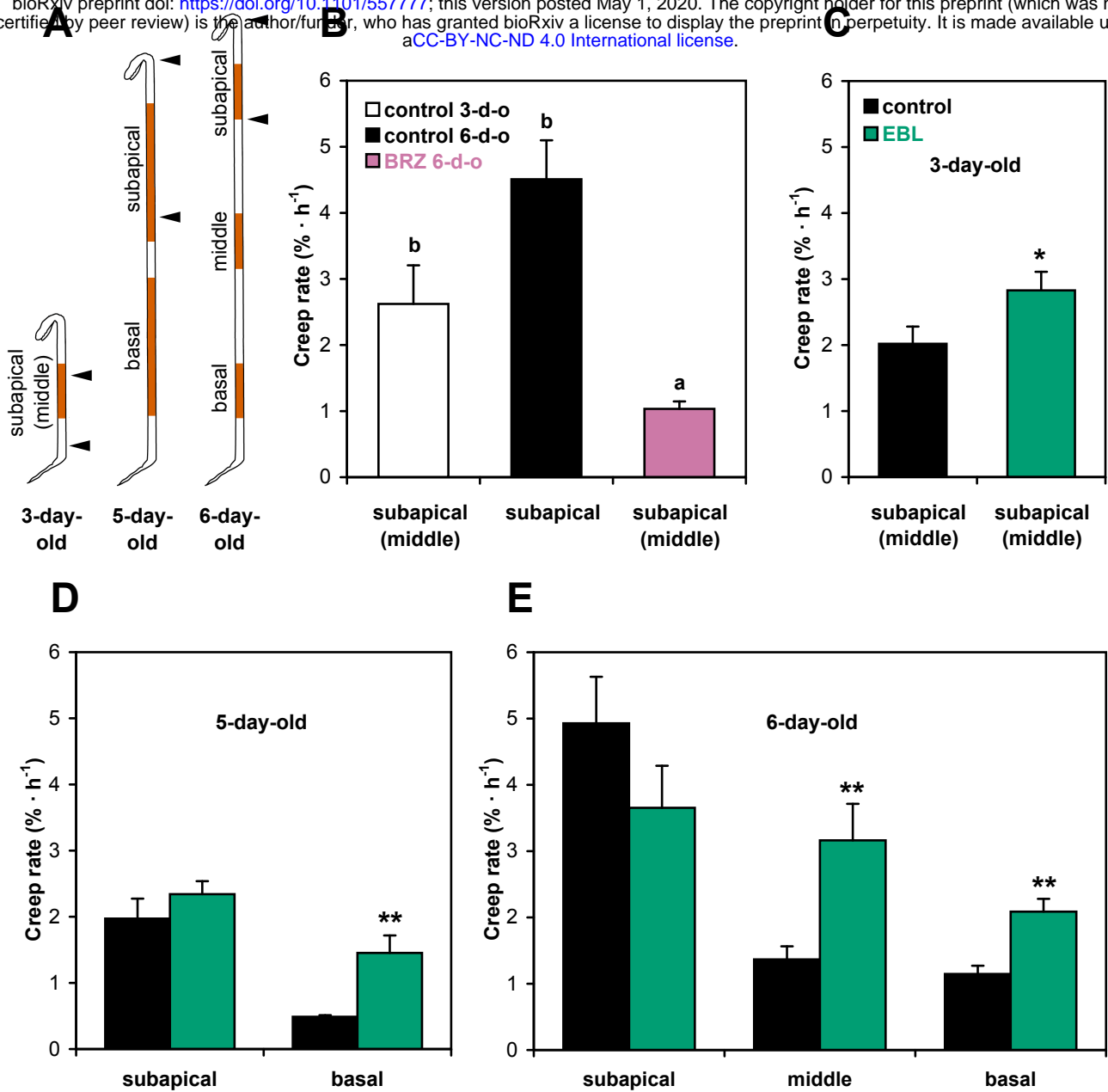
767 **Fig. 5** Cellulose orientation affects the percentage of standing Arabidopsis hypocotyls. *Col-0* seedlings  
768 were grown in darkness on horizontal Petri plates for 5 days without supplements (white bar), with 250  
769 nM oryzalin (orange bar), in each case with 1  $\mu$ M BRZ (hatched bars) or without (solid color bars).  
770 Data are means  $\pm$ SE (n=6). Different letters denote the significant difference between control and  
771 oryzalin-grown plants ( $P=0.0017$ ; Student's *t*-test). Asterisks indicate significant effects of BRZ in  
772 control or oryzalin-grown seedlings (\*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; Student's *t*-test).

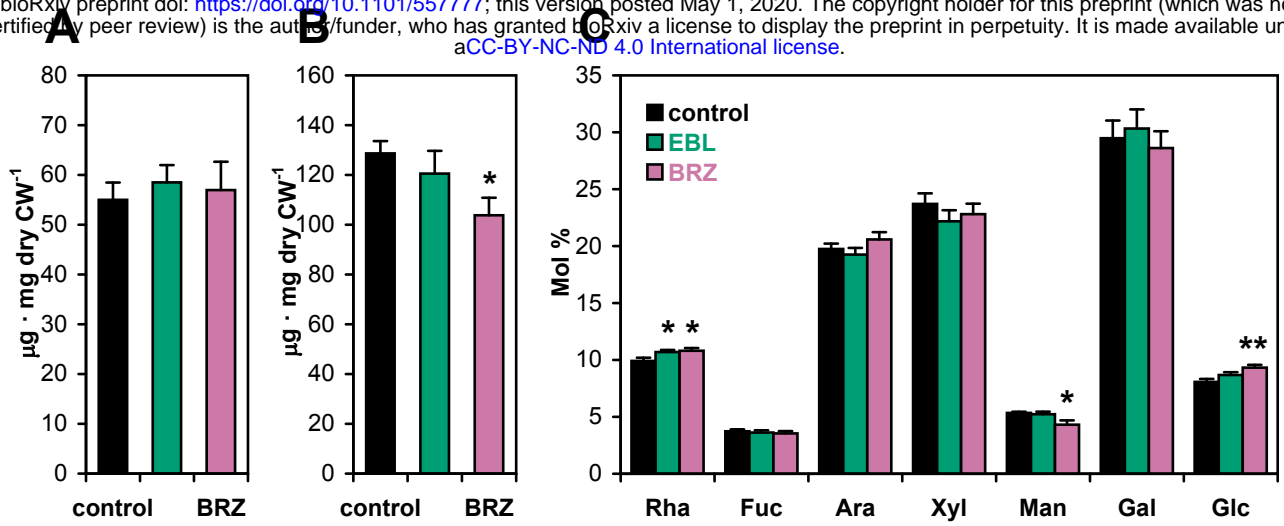
773 **Fig. 6** Hypocotyls of glucomannan-deficient *cs/a2cs/a3cs/a9* mutant plants demonstrate increased  
774 gravitropic bending. Etiolated *Col-0* and *cs/a2cs/a3cs/a9* mutant seedlings were grown on vertical Petri  
775 plates with or without 1  $\mu$ M BRZ for 3 days, subsequently gravistimulated by a 90-degree clockwise  
776 rotation of the plates, and gravitropic bending of their hypocotyls was followed by an infrared imaging  
777 system for 12 h. Data are means  $\pm$ SE (n=10). Asterisks denote significant differences (\* $P$  < 0.05; \*\* $P$  <  
778 0.01; Student's *t*-test) between *cs/a2cs/a3cs/a9* and *Col-0* plants.

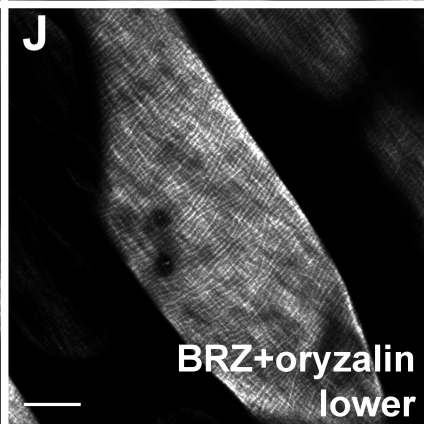
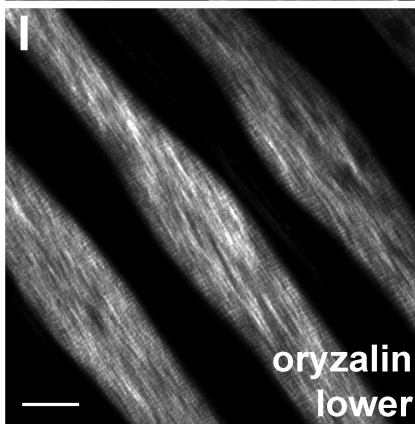
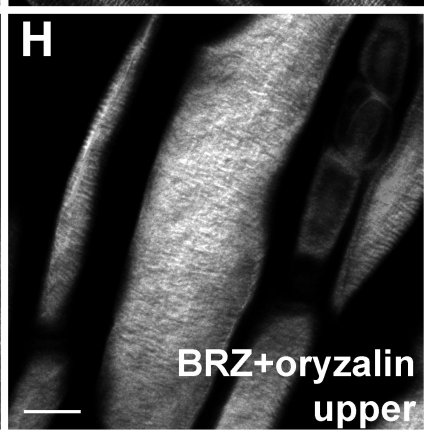
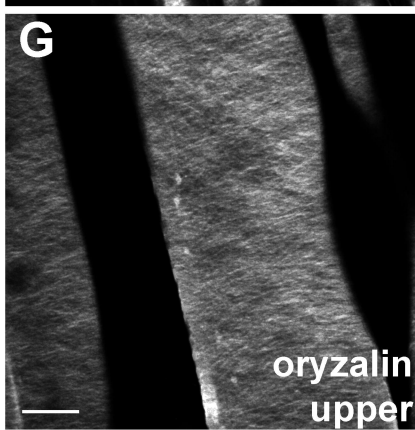
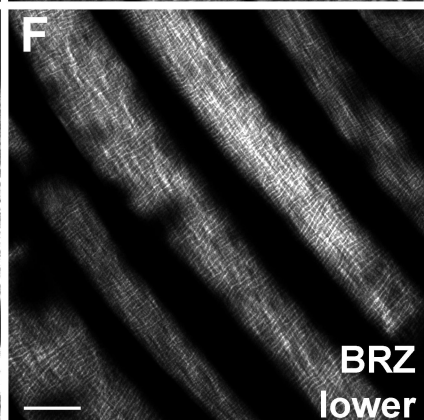
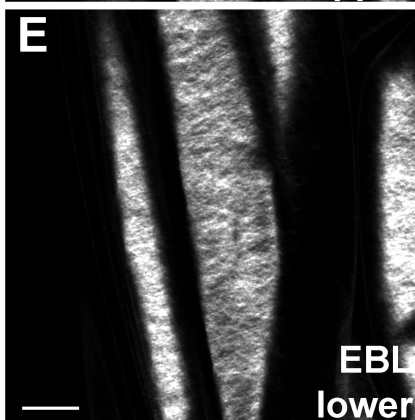
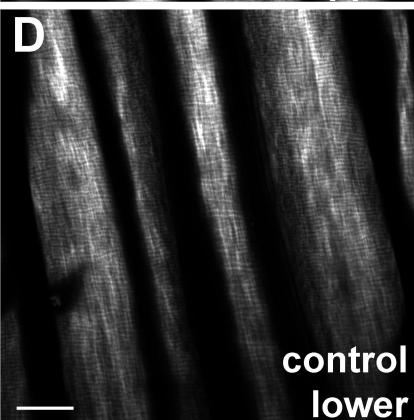
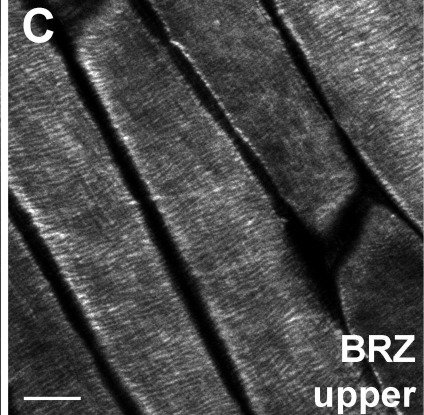
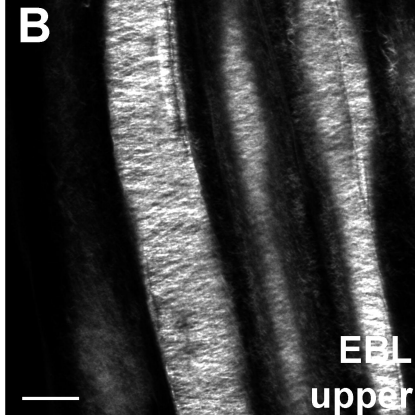
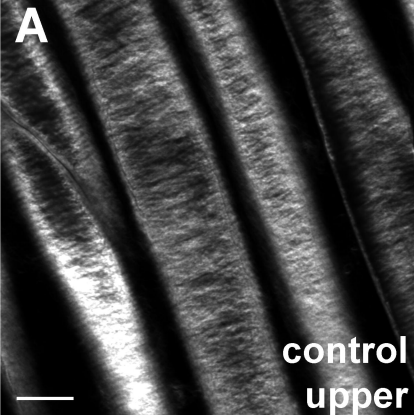
779 **Fig. 7** BRZ inhibits cell expansive growth at the base of Arabidopsis hypocotyls. (A) Epidermal cell  
780 length distribution along the lower parts of hypocotyls in 55- and 72-hour-old etiolated *Col-0* seedlings  
781 grown on horizontal Petri plates with 1  $\mu$ M BRZ or without (control). Cells are numbered from the base  
782 of hypocotyls towards cotyledons. Data are means  $\pm$ SE (n=9). All the cells are significantly shorter in  
783 the presence of BRZ than the respective control cells ( $P$  < 0.01; Student's *t*-test) with the exception of  
784 cells 1 in 55-hour-old plants (not shown on the plot A). (B) Average cell expansion rates at the base of  
785 hypocotyls calculated from the data of plot A (means  $\pm$ SE, n=9). Asterisks mark significant differences  
786 (\* $P$  < 0.05; \*\* $P$  < 0.01; \*\*\* $P$  < 0.001; Student's *t*-test).  
787

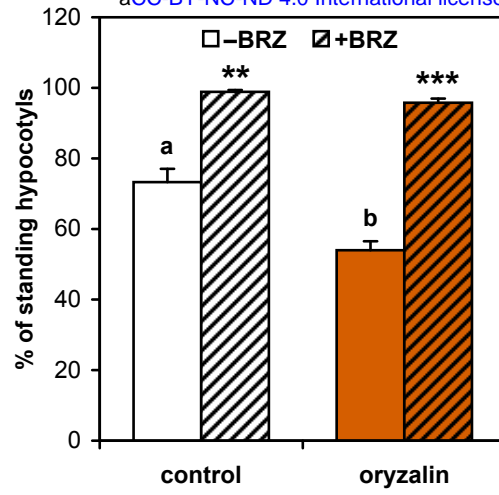


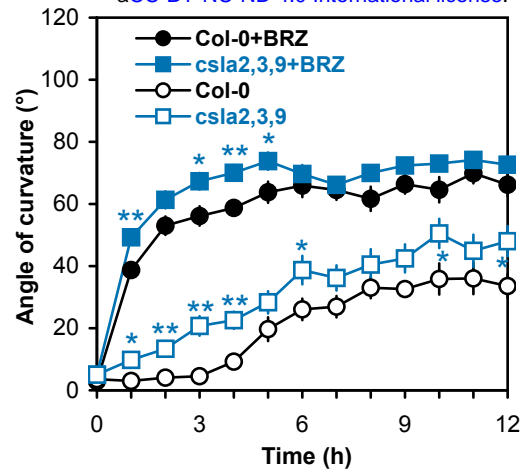




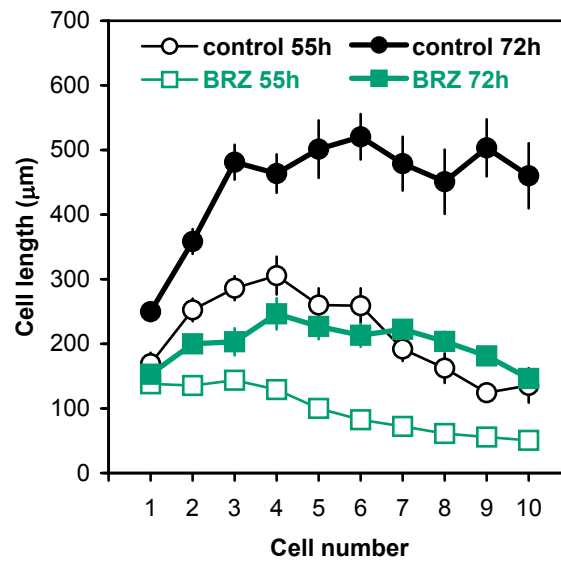








**A**



**B**

