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1 # Dynamic cell wall modifications in brassicas
2 # during clubroot disease

3

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15 **Abstract**

16 Biotic interactions of plants and microbial pathogens can cause drastic changes in cell wall
17 composition in response to developmental reprogramming caused as consequence of an
18 infection. Clubroot disease, caused by the biotrophic plant pathogen *Plasmodiophora brassicae*
19 (Phytomyxea, Rhizaria), is the economically most important disease of Brassica crops
20 worldwide. The disease is best known by the characteristic hypertrophied roots (root galls,
21 clubroots). Amongst a series of physiological changes of the host tissue, the formation of the
22 characteristic root galls leads to cell wall modification and reorganization. Cell wall chemistry
23 and the hosts genetic repertoire are discussed to play a role in the resilience of plants against
24 clubroot disease. Plant cells infected with *P. brassicae* are markedly enlarged, and look very
25 differently from uninfected, healthy cells. Here we systematically review cell wall related
26 processes that lead to the typical clubroot phenotype and provide novel insights how *P.*
27 *brassicae* uses these modifications to benefit its own development. An infection with *P.*
28 *brassicae* impacts on nearly all cell wall related processes, but all alterations are meaningful for
29 successful growth and development of *P. brassicae*. Processes related to cell wall stability and
30 rigidity (e.g. cellulose, pectin or lignin synthesis) are down-regulated, while cell wall degrading
31 enzymes or processes that increase the flexibility of the host cell wall (e.g. expansin) are up-
32 regulated. The here presented findings indicate that *P. brassicae* weakens the structural stability
33 of its host cell while it increases its elasticity, which in consequence allows *P. brassicae* to
34 grow bigger and ultimately to develop more resting spores. Consequently, the understanding of
35 the modification of the host cell wall is important for the formation of the characteristic root
36 galls but also to better understand clubroot disease.

37

38 **Keywords:** *Plasmodiophora brassicae*, *Brassica oleracea*, clubroot, biotic interaction, cell
39 wall, cell wall modification, microbial plant pathogens

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40 # Introduction

41 Upon infection with the obligate biotrophic plant parasite *Plasmodiophora brassicae*
42 (Phytomyxea, Rhizaria) marked morphological changes of the host cells occur. After infection
43 the plant cells start to increase in size and start to alter their shape, which within a few weeks
44 usually leads to gall formation in the roots (Kämper et al., 2006). These galls are the typical
45 symptoms of clubroot disease (Figure 1). This morphological change goes inevitably hand in
46 hand with marked changes in the transcriptomic profile of the host (Zhao et al., 2017;Irani et
47 al., 2018;Olszak et al., 2019). Gall formation in the host is a common feature of phytomyxid
48 parasites: 19 of the 42 phytomyxid species induce hypertrophies in their hosts (Neuhauser et
49 al., 2011;Murúa et al., 2017). The induction of hypertrophies is not limited to plant hosts but
50 can also be found in brown algae and oomycetes (Neuhauser et al., 2014). This formation of
51 galls on the host is linked to the intracellular development of the phytomyxids: gradually larger
52 areas of the host are infected, and multinucleate plasmodia develop and fill their host cells
53 during growth. Phytomyxid growth and development goes hand in hand with developmental
54 reprogramming of the host. Aim of this reprogramming is to generate a sink for nutrients and
55 to create more space for the phytomyxid to grow and ultimately to produce as many resting
56 spores as possible. Here we will discuss the developmental reprogramming of the host
57 associated with the brassica cell wall in clubroot infected plants.

58 The plant cell wall mainly consists of polysaccharides and structural proteins (Figure 2A). The
59 polysaccharides cellulose, hemicelluloses and pectin, are (in varying proportions) the major
60 building blocks of plant cell walls. The currently accepted concept of cell wall structure
61 describes the cell wall as (i) a reinforcing net like structure ("fishing net") of cellulose
62 microfibrils, which are held together by (ii) xyloglucans that bind to the net in clusters
63 stabilizing it and (iii) a pectin gel consisting of stabilized, rigid parts (Ca^{2+}) and very loose
64 pectin gel structures (Cosgrove, 2016).

65 Every plant cell has a primary cell wall, while secondary cell walls are formed in specialized
66 areas of the plant. Primary cell walls are dynamic, growing structures. They are very dynamic
67 in young cells and during cell division. The main function of primary cell walls is the protection,
68 flexibility, basic structural support of the cytoplasm, but they also play a key role in the
69 interaction between cells (Houston et al., 2016). Primary cell walls consist of carbohydrate-
70 based polymers like cellulose, hemicellulose, pectins and glycoproteins (Cosgrove,
71 2005;Sarkar et al., 2009). Primary cell walls are relatively thin, flexible and strong in order to
72 withstand growth related, high turgor pressure, to hold wall stress relaxation and to enable cell
73 elongation (Hamant and Traas, 2010). Newly synthesized wall polymers become integrated by
74 crosslinking, physical interactions or via enzymes. Once plant cells mature and once they have
75 finished expansion and growth, the secondary cell wall biosynthesis is initiated resulting in a
76 remodeled biosynthetic machinery (Nakano et al., 2015). In contrast to the primary cell wall,
77 the secondary cell wall compounds are cellulose, hemicelluloses (mostly xylans) and lignin
78 (Cosgrove, 2005;Sarkar et al., 2009). The secondary cell wall is thicker and is formed at a later
79 stage of plant development. Secondary cell walls mainly provide rigidity and allow for upright
80 growth (Houston et al., 2016). Compared to the primary cell wall, they provide tensile strength
81 rather than extensibility (Speck and Burgert, 2011).

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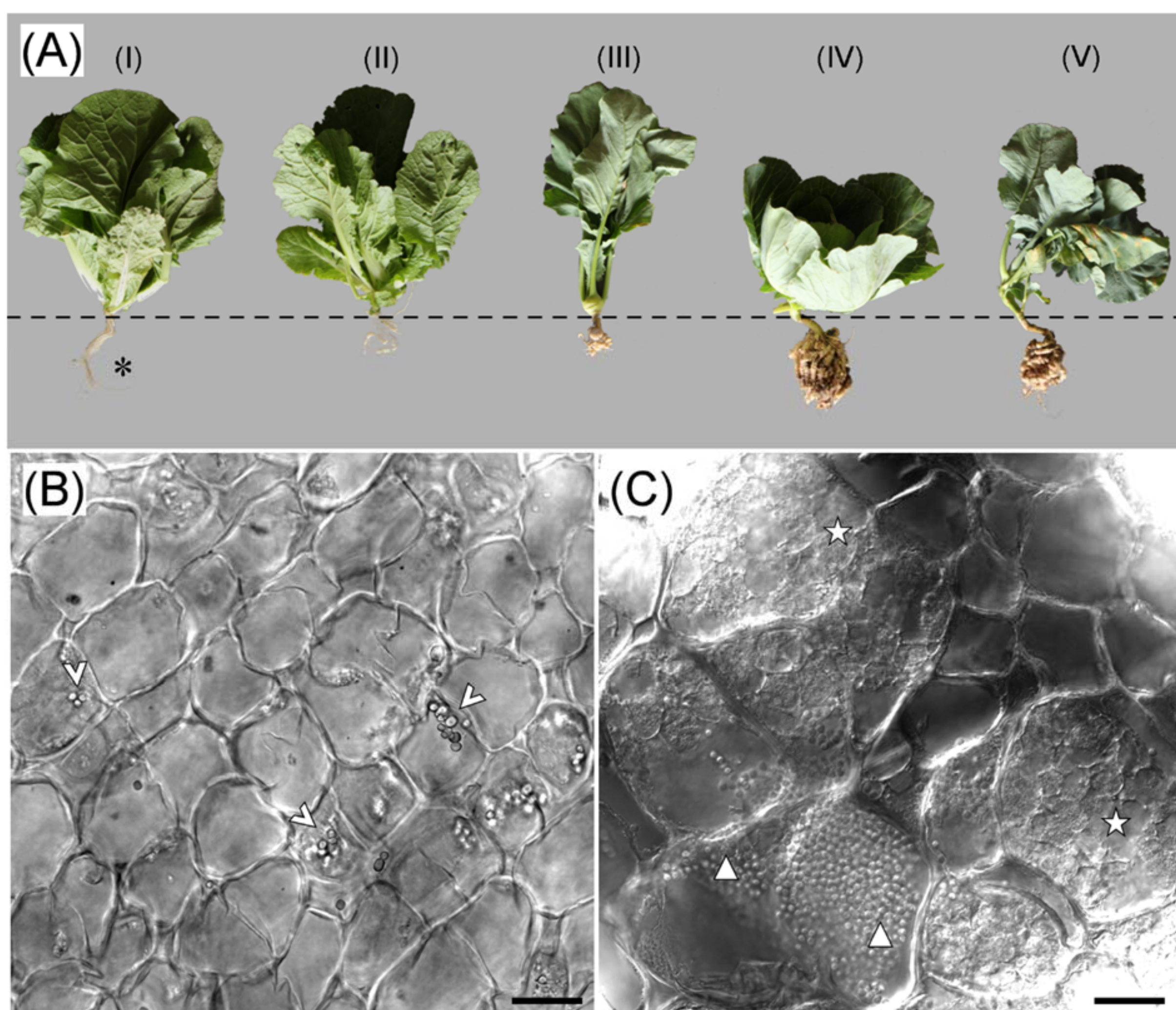

82

83 **Figure 1: Clubroot disease in *Brassica* species.** A: Typical clubroot symptoms caused by *Plasmodiophora*
84 *brassicae*. Above ground development of infected plants is impaired while the roots show the typical
85 hypertrophies. *Brassicae* spp. with and without (*)c clubroot symptoms. (I), (II) *Brassica rapa* subsp. *pekinensis*
86 (Chinese cabbage); (III) *Brassica oleracea* var. *gongylodes* (kohlrabi); (IV) *Brassica oleracea* var. *capitata*
87 (cabbage); (V) *Brassica oleracea* var. *italica* (broccoli). The dashed line indicates the soil surface. B: Section
88 through a healthy *B. rapa* root. Starch granules are indicated with arrowheads. C: Section through a root gall of
89 infected *B. rapa*. *Plasmodiophora brassicae* plasmodia are marked with stars and resting spores with triangles.
90 Scale bar: 30 µm.

91

92 The water insoluble carbohydrate cellulose is the main building block of both, primary and
93 secondary, plant cell walls. Cellulose forms the load-bearing structure of the cell wall, where
94 its fibrous structure is responsible for structural integrity (Maleki et al., 2016). Cellulose is
95 composed of β-1,4-linked glucan chains, which are organized by the cellulose synthase
96 complex (CSC), located in the plasma membrane, and which crystallize to microfibrils (Desprez
97 et al., 2007). The family of cellulose synthase genes (CesA, belonging to the glycosyltransferase
98 family 2, GT2) are the best studied genes contributing to cell wall synthesis, probably because
99 they are present in a multitude of eukaryotes and prokaryotes (Popper et al., 2011).

100 Cellulose is synthesised by the cellulose synthase complex which is located in the plasma
101 membrane and made of several cellulose synthase (CesA) subunits (Jones et al., 2016) and a

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102 sucrose synthase (SuSy), which breaks down sucrose and generates UDP-Glucose (Figure 2A).
103 Different isoforms of cellulose synthase proteins are responsible for the synthesis of cellulose
104 during primary and secondary cell wall synthesis. CesA1, CesA3, and the CesA6-related
105 proteins CesA2, CesA5, CesA9 are involved in primary cell wall cellulose synthesis, while
106 CesA4, CesA7, and CesA8 proteins are responsible for secondary cell wall cellulose synthesis
107 (Desprez et al., 2007;Li et al., 2016). CesAs which are involved in secondary cell wall
108 biosynthesis, also confer resistance against bacterial and fungal pathogens. It is important to
109 note that this resistance pattern is independent of salicylic acid, ethylene and jasmonic acid
110 signaling (Hernandez-Blanco et al., 2007). It is suggested, that CesA1, CesA3 and CesA6 are
111 present in the same plasma membrane protein complex responsible for cellulose biosynthesis,
112 whereas CesA2, and CesA5 (related to CesA6) have partially redundant roles (Desprez et al.,
113 2007). Mutation of any of these primary and secondary cell wall cellulose synthesis genes
114 (CesA1, -3, -6 and CesA4, -7, -8) lead to modifications in composition and structure of both
115 types of cell wall (Hernandez-Blanco et al., 2007;Kumar et al., 2018).

116 The cellulose synthase superfamily also contains nine cellulose synthase-like (Csl) families
117 (CslA - CslJ). The Csl genes share similarities in sequence with the CesA genes and might be
118 involved in the synthesis of the backbone of various polysaccharide polymers (Richmond and
119 Somerville, 2000) like found e.g. in hemicelluloses of the plant cell wall (Lerouxel et al., 2006).
120 Hemicelluloses include polymers like xyloglucans, mannans, glucomannans, and xylans and
121 have β-1,4-linked backbones with an equatorial configuration (Scheller et al., 2010). Unlike
122 cellulose, which is synthesized in the cell membrane, hemicelluloses and pectins are
123 synthesized by glycosyltransferase (GTs) in the golgi apparatus (Suzuki et al., 2006) from
124 where the native hemicellulose/pectins are transported to the membrane with vesicles. They are
125 then secreted to the apoplast and enzymatically incorporated into the cell wall (Verbancic et al.,
126 2018) (Figure 2B).

127 Pectins are the most heterogeneous and complex group of polysaccharides of the cell wall and
128 mainly occur in the primary cell wall. Pectins found in the plant cell wall are homogalacturonan
129 (HG), rhamnogalacturonan I (RGI) and II (RGII), and xylogalacturonan (XGA). Together with
130 hemicelluloses they form the matrix in which cellulose microfibrils are embedded. Pectins play
131 important roles for cell expansion, adhesion, strength, and porosity, but are also relevant for
132 signaling between cells (Harholt et al., 2010;Ferrari et al., 2013). Together with other cell wall
133 forming polymers, pectin is involved in providing physical strength to the plant. Plant
134 pathogens can cause degradation of pectin by breaking it down into oligogalacturonides (OGs),
135 which are involved in signaling for sensing pathogen caused wall degradation (Ferrari et al.,
136 2013) (Figure 2B).

137 The deposition of lignin in the polysaccharide matrix during maturation of the secondary cell
138 wall is typically the final step of secondary cell wall biosynthesis (Meents et al., 2018). It
139 ensures two characteristic properties of the secondary cell wall: increased rigidity and
140 impermeability. Lignin biosynthesis can be induced during cell development and maturation.
141 But lignification of the plant cell wall can also be a response to biotic and abiotic stress, for
142 example to protect other cell wall polysaccharides from degradation (Tronchet et al., 2010).

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143 Plant cell walls are very dynamic, and responsive systems and can be modulated and remodeled
144 by the plant itself (e.g. cell growth, elongation, reinforcement) or by pathogens (e.g. degrading
145 enzymes (Underwood, 2012)). When *P. brassicae* infects the root cells of its host, those cells
146 show a marked cell expansion, what differs significantly from cell growth in symptomless roots.
147 Generally growth or an increasing size of plant cells is accompanied by the enlargement of the
148 cell caused by an increase of turgor inside the plant which is generating cell wall stress
149 (Cosgrove, 2016). This cell wall stress is relaxed by cell wall loosening processes (including
150 xylanases, expansins etc) after a certain tipping point. These structural changes in the cell wall
151 allow rapid expansion, which then again results in reduced turgor and the possibility for water
152 to permeate the cell. This is followed by growth of the cell and by an increase of cell wall
153 material. Cell wall stress is subsequently followed by reinforcement of the now "loose" cell
154 wall through structural cell wall components. Enzymes like cellulases, Xyloglucan
155 endotransglycosylases/hydrolases (XTHs) and expansins are mainly involved in wall
156 expansion. They are supposed to disrupt noncovalent linkages between wall polysaccharides
157 (Cosgrove, 2005) (Figure 2B).

158 Plant cell wall degrading enzymes (CWDEs) play an inevitable role for both, plants and
159 pathogens. In plants they are involved in cell wall dynamics, e.g. in loosening, breakdown and
160 the deposition of newly synthesized wall components (Marin-Rodriguez et al., 2002). They are
161 also important for infection and establishment of plant pathogens (Kämper et al.). De-
162 polymerization causes modification and alteration in wall rheology (Figure 2B). However,
163 biotrophic pathogens like *P. brassicae* generally encode few CWDEs to avoid their recognition
164 by the plant host (Kämper et al., 2006;Schwelm et al., 2015).

165

166 # Materials and Methods

167 Cell wall related genes were extracted from an existing *Brassica oleracea* var. *gongylodes*
168 RNA-seq dataset (Ciaghi et al., 2019) were used (Accession: European Nucleotide Archive
169 (ENA; https://www.ebi.ac.uk/ena) under the project PRJEB26435). These RNAseq data
170 originated from white spindle galls and brownish spindle galls. Relevant pathways related to
171 cell wall biosynthesis, rigidity, modulation, elongation, growth etc. were analyzed using all
172 available information. Plant genes of interest (i.e. genes related with cellulose, hemicellulose,
173 pectin, and lignin metabolism and cell expansion) were identified in this data using automatic
174 gene annotations provided by MapMan (Thimm et al., 2004), KAAS (KEGG Automatic
175 Annotation Server; Moriya et al., 2007), and eggNOG mapper (Huerta-Cepas et al., 2016).
176 Transcripts fulfilling the criteria were curated manually. In general, only significantly
177 differentially expressed genes (DEGs, adjusted p-value < 0.05) were analyzed but very rarely
178 non-significant DEGs were included where it was useful for the biological interpretation and
179 discussion of the data (noted in the text). This approach was chosen, because in a complex
180 dataset like clubroots that contain a multitude of physiologically and developmental different
181 cells smaller changes might still be relevant for a biological discussion of the plant pathogen
182 interaction and for the formulation of a biological hypothesis. Log_2-fold change values of
183 significant DEGs (infected vs. control) and their corresponding FPKM (fragments per kilobase
184 per million reads) values were used for this study, without any further threshold values.

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185 MapMan, KAAS, eggNOG and TAIR Blast annotations were compared and genes grouped by
186 those automated annotations. Putative isoforms were validated using alignments of the amino
187 acid and protein sequence when contradictive expression values of DEGs were found. Isoforms
188 were weighted based on their length, on sequence similarity and based on the general expression
189 trend of all isoforms. Additionally, results published studies on clubroot disease were integrated
190 to provide a comprehensive overview on cell wall related changes in clubroot infected plants.

191

192 Results

193 Synthesis of cell wall components

194 Cellulose synthase (CesA) and cellulose synthase-like (Csl) gene expression

195 Transcripts of the cellulose synthases CesA1-6 were identified in our data (Tab. S1). All of
196 them were significantly down-regulated in clubroots compared with the control. CesA1-A6
197 transcripts were down-regulated during clubroot development.
198 Cellulose synthase-like A2 (CslA2) transcripts (Tab. S2) were significantly up-regulated in
199 clubroots compared with the control, while CslA (Csl A3, 7, 11) transcripts were down-
200 regulated, although this down-regulation was not statistically significant. CslA genes are
201 involved in the beta-mannan biosynthesis, while CslC genes are supposed to be involved in
202 xyloglucan synthesis. Of the CslC gene family transcripts belonging to CslC4, 5, 6, 8, 12 were
203 identified in our dataset. Compared with the control, CslC4 and CslC6 was down-regulated in
204 clubroots while CslC5 genes were up-regulated (Tab. S2). CslD genes are involved in the xylan
205 and mannan biosynthesis and CslD3 were significantly down-regulated while CslD2, 5 were
206 up-regulated. From the CslE family only CslE1 transcripts were significantly up-regulated
207 clubroots.

208 To summarize: cellulose synthases (CesA family) were down-regulated in clubroots, whereas
209 cellulose synthase-like genes (Csl) presented a more ambiguous picture, where some genes
210 were up-regulated (CslA2, CslC5, CslD2, CslD5, CslE1) while others were down-regulated
211 (CslA3, CslA7, CslA11, CslC4, CslC6, CslD3) in clubroot infected plants.

212 Pectin, lignin and callose synthesis genes

213 In clubroot infected tissue, transcripts linked to the synthesis of the most abundant pectins were
214 down-regulated. Rhm1, Rhm2, and Quasimodo1 (Qua1) are coding for enzymes involved in
215 the synthesis of rhamnose I (monosaccharides), and are involved in the homogalacturonan
216 (polysaccharide) synthesis, two of the major components of pectin. All three genes were
217 significantly down-regulated in clubroot tissue (Tab. S3).

218 Genes coding for an isoform of UDP-glucoronic acid decarboxylase (UXS), which produces
219 UDP-xylose as a substrate for cell wall carbohydrates like pectin and hemicelluloses, were up-
220 regulated (UXS1, UXS3, UXS5) and UXS2 was down-regulated in clubroot infected plants
221 (Tab. S3).

222 In clubroot tissue, the genes involved in lignin biosynthesis were generally down-regulated in
223 roots infected with *P. brassicae* (Tab. S4). The first three steps of the phenylpropanoid pathway,

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224 which initiates lignin biosynthesis are mediated through PAL (phenylalanine ammonia lyase),
225 C4H (cinnamate 4-hydroxylase), and 4CL (4-coumarate:CoA ligase). Transcripts belonging to
226 these gene families were down-regulated in clubroot infected tissue. Other lignin biosynthesis
227 genes like COMT (caffeic acid O-methyltransferase), CCR (cinnamoyl-CoA reductase), and
228 F5H (ferulate 5-hydroxylase) were also down-regulated.

229 Laccases and peroxidases are involved in the monolignol dehydrogenation step during lignin
230 biosynthesis. With few exceptions genes coding for laccases and peroxidases involved in lignin
231 synthesis were down-regulated in clubroot infected plants (LAC1, LAC3, LAC7, LAC11,
232 LAC17). LAC6 and LAC13 were up-regulated.

233 Glucan-synthase-like genes (GSL), involved in callose biosynthesis, were generally down-
234 regulated in clubroot tissue (GSL3, GSL7, GSL8, GSL10, GSL12) (Tab. S5). All these genes
235 encode callose synthase (CalS). Genes coding for CalS1, the catalytic subunit 1 of callose
236 synthase were up-regulated. UGT1 (UDP-glucose transferase 1), which is also involved in
237 callose synthesis (transport of UDP-glucose to CalS), was down-regulated.

238

239 **2. Degradation and modification of components**

240 **Cell wall degrading and modifying enzymes and inhibition of cell wall degradation.**

241 The most abundant transcripts of cell wall degrading enzymes found in clubroot infected plant
242 tissue were pectinases and cellulases. Transcripts belonging to pectin degrading enzymes of the
243 polygalacturonases (PGs), pectate lyases (PLs), pectin methylesterases (PMEs), and PAEs
244 (pectinacetylesterases) were mostly up-regulated (some of them strongly) in infected roots
245 (Tab. S7). Transcripts belonging to glycoside hydrolase family 28 proteins (GH28) were up-
246 regulated in clubroots. All genes coding for PMEs (like PME3, PME 5, PME31), and most
247 genes coding for PAEs (like PAE7, PAE 9-11) were up-regulated in infected plants, only PAE5
248 and PAE12 were down-regulated. Genes coding for callose degradation, or deposition were
249 also up-regulated in clubroots (Tab. S5, S8).

250 Cellulases with hydrolase activity were generally up-regulated in clubroots, including glycosyl
251 hydrolases family 9 (endoglucanases) like GH9B1, 6-8, 12, 13, GH9A1, and GH9C3. GH9B1,
252 -13, GH9A1 and GH9C3 were down-regulated in root galls. Some genes of the glycosyl
253 hydrolases family 17 were up-regulated in clubroots. Beta glucosidases were up- or down-
254 regulated in clubroots (Tab. S8).

255 Inhibitors of cell wall degrading enzymes like polygalacturonases (PGs) and pectin
256 methylesterases (PMEs), which dynamically modulate PMEs and PGs to inhibit pectin
257 depolymerisation were down-regulated in clubroots (Tab. S9). Genes coding for PGIP2
258 (polygalacturonase inhibitor protein 2) were strongly down-regulated. PGIP1 were mainly
259 down-regulated in infected roots. Genes coding for PMEIs (pectin methylesterase inhibitors)
260 were also mainly down-regulated in clubroots, for example like PMEI6, -11 and PMEI13. Root
261 hair specific 12 (RHS12) with pectin methylesterase inhibitor activity was also down-regulated
262 in clubroots.

263

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264 **3. Cell wall loosening, elongation**

265 ***P. brassicae* infection causes cell wall loosening in plant roots**

266 Genes involved in cell wall loosening and elongation processes like xyloglucan
267 endotransglucosylases/hydrolases (XTHs) or expansins (EXP) and expansin-like genes (EXL)
268 were mainly up-regulated (Tab. S10, S11). (XTH4, -9, -16, -24 and -32, EXP1, -6, -13, -15 and
269 -20, EXPB2, and -3, EXLB1). In contrast genes like XTH 8, -10, and XTH21 and EXLA1, -2,
270 and EXP 3 and EXP17 were down-regulated, in clubroots.
271 Wall associated kinases (WAKs) and wall associated kinase-like genes (WAKLs) are also
272 involved in cell elongation, and all WAKs were up-regulated, whereas WAKLs were down-
273 regulated in clubroots (Tab. S12).
274 Genes coding for SHAVEN3 (SHV3) and SHV3-like (SVL1) are also involved in cell
275 expansion and alteration of wall rigidity. They were both up-regulated clubroots (Tab. S13).

276

277 # Discussion

278 ## *Plasmodiophora brassicae* induces alterations of host cell wall rigidity

279 An infection with the phytomyxid *Plasmodiophora brassicae* results in molecular and
280 physiological changes in the plant host: abnormal root growth results in gall formation. This is
281 accompanied by altered metabolism, physiology and modifications of the host cell walls
282 (Dekhuijzen and Overeem, 1971; Kavanagh and Williams, 1981; Ludwig-Müller et al., 1993;
283 Devos et al., 2005; Ludwig-Müller et al., 2009; Malinowski et al., 2012). The growth of the
284 host cells is resulting in surface increase, however, this is not automatically coupled to addition
285 of new cell wall components (Cosgrove, 2014a). In clubroot infected cells the biosynthesis of
286 wall components like cellulose, hemicelluloses, pectin (primary cell wall) and also lignin
287 (secondary cell wall), which together form the load-bearing structures in the cell wall, is down-
288 regulated (Tab. S1 – S4). These findings could be also shown in other experiments containing
289 transcription analysis in clubroot infected *A. thaliana* (Irani et al., 2018). Genes involved in
290 lignin biosynthesis are also down-regulated in former studies with infected *B. napus* (Cao et al.,
291 2008; Ludwig-Müller et al., 2009). These results suggest an inhibition of cell wall component
292 biosynthesis in clubroot infected tissue. The observed down-regulation of genes coding for
293 biosynthesis of cellulose synthases (CesA), pectins (RHM, QUA) and hemicellulose (Csl
294 genes) in infected plants supports this finding, which indicates that the cell walls of clubroot
295 infected cells are less rigid than their counterparts in uninfected plants. Also transcripts of genes
296 coding for cell wall degrading enzymes (CWDEs) like pectinases [polyglacturonases (PGs),
297 pectate lyases (PLs) and pectin methylesterases (PMEs)] and cellulases with hydrolase activity
298 were up-regulated (some strongly) in infected plants (Tab. S7, S8). These findings could also
299 be validated in other studies of early infection of *B. oleracea* infected with *P. brassicae* (Wang
300 et al., 2019).
301 In other words, *P. brassicae* is responsible for a decrease in root cell wall rigidity and stability
302 in clubroots via down-regulation of the biosynthesis of primary and secondary cell wall
303 components like cellulose, pectin, callose or lignin. On the other hand, enzymes which degrade
304 these structural elements are up-regulated in clubroots. This loss in stability does fit the
305 biological development of *P. brassicae*, because the multinucleate plasmodia gradually fill the

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306 significantly enlarged host cells. By reducing the rigidity of the cell wall, *P. brassicae* can grow
307 to a larger size as the barrier posed by the cell wall gets more flexible. The processes seen at
308 the transcriptomic level resemble the processes described during wall stress related growth as
309 processes that control rigidity, but also extensibility is differentially regulated - with the main
310 aim to withstand increasing turgor pressure without rupture of the cell (Cosgrove, 2016), while
311 increased turgor-like pressure is built up by the growing parasite. That decreased rigidity of the
312 cell wall has an important role for clubroot symptom development. This is supported by the
313 finding that clubroot tolerant plants show transcriptomic signatures of an increased cell wall
314 stability (Lahlali et al., 2017). The limited arsenal of CWDEs of *P. brassicae* has been notable
315 when the genome of this parasite was analyzed (Schwelm et al., 2015;Rolfe et al., 2016). A lack
316 of CWDEs is on the one hand a feature that is seen in many biotrophic plant parasites (Kämper
317 et al., 2006). But on the other hand, the transcriptional changes observed in clubroot suggest
318 that *P. brassicae* is able to compensate this by reprogramming the host metabolism.

319

320 In addition to a reduced rigidity of the plant cell wall strength, depolymerized pectin can serve
321 as carbon source for pathogen development (Jia et al., 2009) and increases the accessibility of
322 other plant cell wall components like cellulose and hemicelluloses to CWDEs, which can enable
323 further and faster cell wall breakdown (Xue et al., 2018). This again would tie in very well with
324 the biology of *P. brassicae,* which is obtaining nutrients from its host. So an increased
325 availability of cell wall degradation products (sugars) can provide energy for *P. brassicae*. In
326 order to protect the plant cells from excessive degradation of wall components through
327 pectinases like PMEs or PGs, the host is able to produce enzyme-inhibiting enzymes, called
328 PMEIs (PME-inhibitor) and PGIPs (PG-inhibiting protein). These enzymes modulate PMEs
329 and PGs dynamically, and genes coding for these inhibitors were all down-regulated in clubroot
330 tissue (Tab. S9). So as expected from a biotroph, *P. brassicae* induces changes in its host via
331 inhibiting a cell wall related plant defense mechanism.

332 Plant genes involved in the metabolism of sugars that are building blocks of cell wall
333 components were up-regulated in infected roots (Tab. S6). Transcripts of genes coding for
334 sucrose synthase (SuSy), which catalyzes the reaction from sucrose to UDP-glucose and
335 fructose are one example. UDP-glucose is needed as substrate for cellulose synthesis using
336 CesA and for callose synthesis using CalS (Schneider et al., 2016) (Figure 2A). Genes coding
337 for UDP-glucuronate decarboxylase (UXS), which produces UDP-xylose, a substrate for
338 hemicelluloses and pectin, are up-regulated in clubroot transcriptomes (Tab. S3). Up-regulated
339 sugar metabolism could also be shown in several other studies of clubroot infected *A. thaliana*
340 (Siemens et al., 2006;Siemens et al., 2011;Walerowski et al., 2018). Summarizing these
341 findings, it can be hypothesized that the increase in plant transcripts coding for CWDEs likely
342 results in increased sugar availability via the degradation of cell wall components. On the other
343 hand, the increase of transcripts belonging to sugar metabolic pathways likely results in an
344 increased production of cell wall related sugars such as UDP-glucose or UDP-xylose. Overall
345 it can be assumed, that these sugars might be taken up by *P. brassicae* as energy source,
346 however, this hypothesis needs to be addressed separately.

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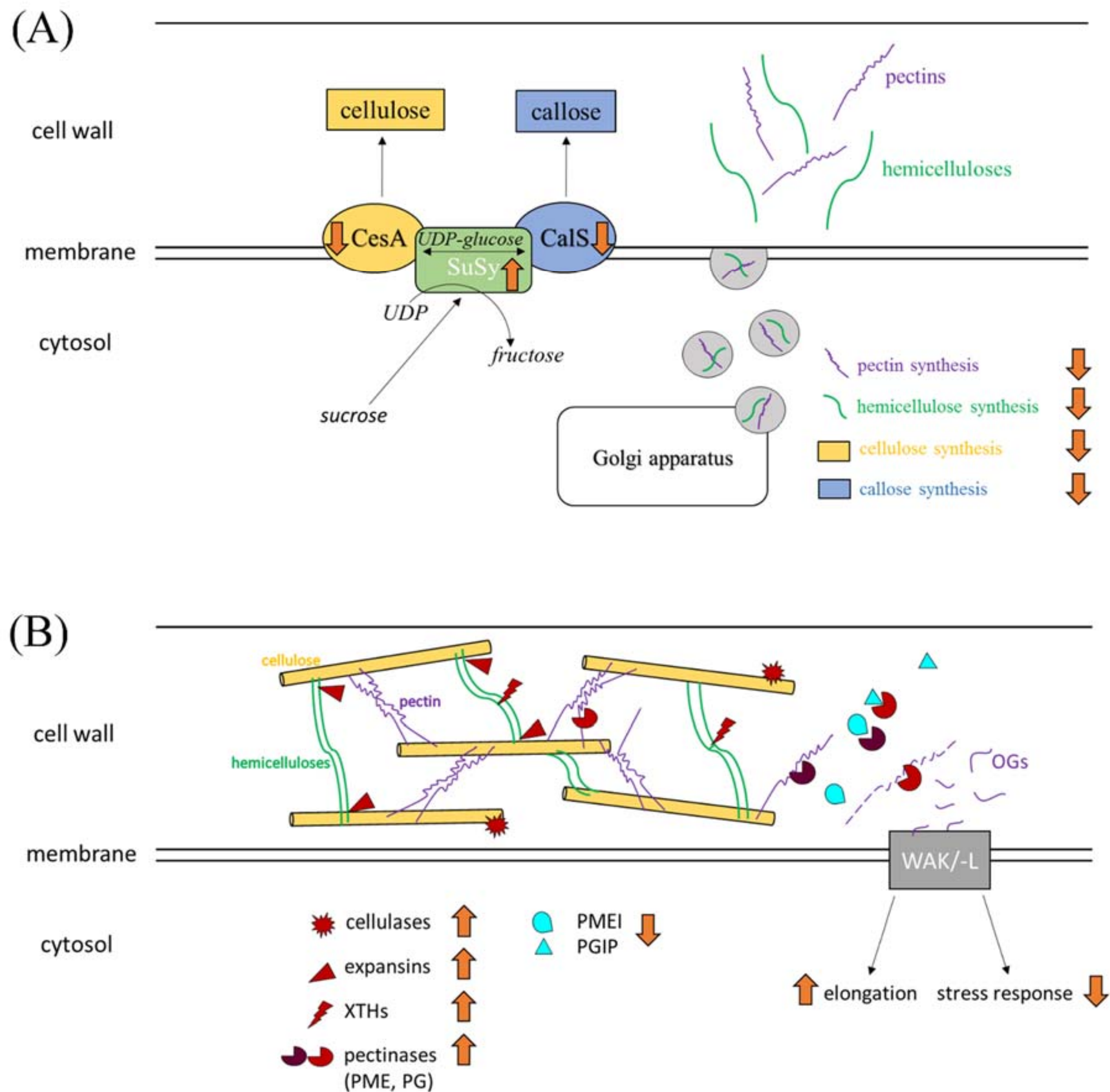

347
348 **Figure 2: Synthesis of plant cell wall components (A) and modulation of cell wall components and related**
349 **plant response (B).** (A): Cellulose and callose synthesis are mediated through cellulose synthase (CeSA and
350 callose synthase (CalS) in the plasma membrane. UDP-glucose serves as substrate for the synthesis. Sucrose
351 synthase breaks down sucrose and generates UDP-glucose and fructose. Pectins and hemicelluloses are
352 synthesized in the Golgi apparatus. Native pectins and hemicelluloses with sufficient length are transported by
353 vesicles, secreted to the apoplast, and enzymatically incorporated into the cell wall. During infection with the
354 biotrophic clubroot pathogen *Plasmodiophora brassicae,* genes involved in the synthesis of cellulose,
355 hemicelluloses, pectins and callose are down-regulated. Genes coding for sucrose synthase are up-regulated in
356 clubroots. Up- and down-regulated processes in clubroots is indicated with orange arrows. (B): Cell wall degrading
357 and modulating enzymes and their target components. Cellulases modulate cellulose fibrils, XTHs cleave and re-
358 anchor xyloglucan chains (hemicellulose), expansins disrupt linkages between hemicellulose and cellulose, and
359 pectinases cleave pectins. Depolymerized pectins (oligoglacturonases, OGs) are bound and recognized by wall
360 associated kinase/-like (WAK/L) and can trigger further plant response cascades. Pectinase-inhibitors (PMEIs,
361 PGIPs) inhibit PME and PG activity. During infection with the biotrophic clubroot pathogen *Plasmodiophora*
362 *brassicae,* genes like cellulases, pectinases (cell wall component degradation) and expansins and XTHs (cell
363 expansion) are up-regulated in infected tissue. Enzyme inhibitors like PMEI or PGIP are down-regulated. Genes
364 coding for WAKs (involved in cell elongation) are up-regulated and for WAKLs (stress response) are down-
365 regulated in clubroots. Up- and down-regulated processes in clubroots is indicated with orange arrows.

366

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367 ***Plasmodiophora brassicae* causes cell elongation and inhibits host defense**
368 **response**

369 To allow for cell expansion, an organized disruption of the existing cell wall homeostasis is
370 followed by reinforcement via the formation of new linkages of wall components. Organized
371 cell wall disruption can happen with the aid of CWDEs as described above, or via the enzymatic
372 modification of wall rigidity and stability with expansins and xyloglucan
373 endotransglycosylases/hydrolases (XTHs). Expansins and XTHs are directly involved and
374 responsible for such modifications (Figure 2B). Transcripts of genes coding for expansins and
375 for XTHs were significantly up-regulated in our data and previous transcriptome profiling
376 works of clubroots (Siemens et al., 2006; Irani et al., 2018) (Tab. S10, S11). XTH activity was
377 also detected in *B. rapa* by Devos et al. (2005). XTHs have two functions, either as xyloglucan
378 endohydrolase (XEH), which cleaves xyloglucan chains, or as xyloglucan endotransglycosylase
379 (XET), which is able to re-anchor two xyloglucan microfibrils, which have ruptured as result
380 of turgor driven cell wall disruption (Rose et al., 2002). The majority of *B. oleracea* genes that
381 were up-regulated in clubroots were annotated as XTHs possessing both functions (cleavage
382 and re-anchoring). Notably, transcripts of genes that only feature the re-anchor activity (XET)
383 were down-regulated. In other words, when *P. brassicae* plasmodia are growing in the infected
384 host cell they might activate certain aspects of turgor driven cell growth in their host (up-
385 regulation of expansins and XTHs with XEH and XET activity), but inhibit the plants ability to
386 re-inforce the cell wall (down-regulation of XTHs with only XET activity). Other genes
387 involved in cell expansion and alteration of wall rigidity with important roles in cell wall
388 organization are genes coding for SHV3 (SHAVEN3) and SVL1 (SHV3-like). *Arabidopsis*
389 *thaliana* SHV3 and SVL1 double mutants resulted in further defects like abnormal cell
390 expansion, bloat guard cells as well as ectopic lignin deposits. Also pectin modifications and
391 altered cellulose contents were observed (Hayashi et al., 2008). Related genes were up-
392 regulated in clubroots (Tab. S7, S8, S13).

393 Wall associated kinases (WAKs) belong to the receptor-like kinases with a cytoplasmic protein
394 kinase domain. They are located in the plasma membrane and serve as pectin receptors (cell
395 wall side), for longer pectins of native cell walls and short pectic fragments of oligogalacturonic
396 acid (OGs) (Kohorn, 2016) (Figure 2B). WAKs do not just bind pectins, they also recognize
397 pectin fragments, which can be produced by pectin-degrading plant pathogens. This gene family
398 is also involved in triggering stress response cascades in the host plant (Sivaguru et al., 2003;
399 Kohorn and Kohorn, 2012). WAK genes are also discussed to be involved in cell elongation,
400 because WAK mutants with lower levels of WAK proteins led to loss of cell expansion (Lally
401 et al., 2001), shorter roots and a reduction in vacuolar invertase what might control expansion
402 indirectly through turgor pressure (Kohorn et al., 2006; Kohorn et al., 2009; Kohorn and
403 Kohorn, 2012). WAK-like genes (WAKL) share sequence similarities with WAKs, but have a
404 direct role in disease resistance at least in maize (Hurni et al., 2015;Zuo et al., 2015). Evidence
405 for this is provided, because dominant mutant allels of WAKLs in *A. thaliana* showed resistance
406 to *Fusarium* (Diener and Ausubel, 2005). In clubroot infected plants, all genes coding for
407 WAKs were up-regulated which fits very well to the above discussed increase in cell expansion.
408 Overall, stress response is down-regulated in clubroots (Zhao et al., 2017), so an influence in
409 stress response is not clear. All genes encoding WAKLs are down-regulated in clubroots, what

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410 might influence resistance negatively of host plants, because WAKLs are supposed to play a
411 role in pathogen resistance (Verica and He, 2002) (Tab. S12).

412 Upon pathogen derived wall damage, the plant immune system can initiate a reinforcement of
413 the cell wall as first line of defense, where a reinforced cell wall acts as physical barrier to
414 infection. In order to slow down or to prevent pathogen invasion, callose or lignin containing
415 cell wall thickenings are formed at infection sites (Stone, 1992). The most notable biochemical
416 difference between clubroot-susceptible and resistant plants is an increased biosynthesis of
417 lignin and phenolics, in the resistant plants (Lahlali et al., 2017). The key precursor of the
418 phenylpropanoid and lignin pathways is L-phenylalanine (Barber and Mitchell, 1997; Shadle
419 et al., 2003). PAL (L-phenylalanine ammonia-lyase) is the entry point enzyme to these
420 pathways and the key regulator of the phenylpropanoid pathway, which can also be induced by
421 pathogens (MauchMani and Slusarenko, 1996; Cozzolino, 2014). PAL and the lignin
422 biosynthetic pathway appear to play an important role in resistance to clubroot conferring to
423 the characterized clubroot resistance gene *Rcr1*. In clubroot resistant varieties of oilseed rape,
424 PAL was highly up-regulated resulting in changes of the cell wall composition and lignification
425 after contact with *P. brassicae* (Lahlali et al., 2017). PAL genes and the other important genes
426 involved in lignin biosynthesis (coding for cinnamate 4-hydroxylase (C4H) and 4-
427 coumarate:CoA ligase (4CL)) were down-regulated (Tab. S4), suggesting *P. brassicae*
428 infection suppresses induced defense and resistance response of the *Brassica* host (Ciaghi et
429 al., 2018).

430 Another possibility of cell wall reinforcement are callose-containing papillae (Voigt, 2014).
431 Increased callose deposition is associated with the clubroot resistance gene *Rcr1* (Chu et al.,
432 2014). Callose is synthesized in specialized cell walls like the cell plate (separates dividing
433 cells) by the callose synthase, which is encoded by CalS genes belonging to the GSL (glucan
434 syanthase-like) gene family. This family is divided in genes involved in fertility and cell
435 division (GSL1, GSL2, GSL6, GSL8, GSL10) and in wall reinforcement (GSL5, GSL7,
436 GSL12) (Verma and Hong, 2001). In our data, many transcripts could be annotated as GSL1,
437 GLS8, GSL10 (fertility and division), and GSL7, GSL12 (reinforcement). All of them were
438 down-regulated in our dataset (Tab. S5). GSL5, also known as powdery mildew resistant 4
439 (PMR4) involved in callose biosynthesis, is known to be re-located in the plasma membrane
440 via vesicle-like bodies to papillae formation sites in *Arabidopsis thaliana* (Flors et al., 2008;
441 Ellinger et al., 2013). GSL5 is (not significantly) down-regulated in our dataset, however, all
442 available data suggest that an infection with *P. brassicae* leads to a decreased callose
443 biosynthesis, deposition of callose synthase to pathogen site and for increased deposition of
444 already synthesized callose, in order to reduce plant defense response. The genes PEN2 and
445 PEN3 (Penetration2, 3) are also involved in callose deposition and glucosinolate activation and
446 therefore necessary for plant resistance (Clay et al., 2009). Genes coding for PEN2 and PEN3
447 are up-regulated in our data, suggesting increased callose deposition and glucosinolate
448 activation (Tab. S5). Glucosinolates are sulfur-containing glucosides and known as essential
449 antimicrobial components in *Arabidopsis thaliana* (Hogge et al., 1988). Microorganisms are
450 inhibited by breakdown products of glucosinolates derived through thioglucosidases
451 (Kirkegaard et al., 1996; Manici et al., 1997; Tierens et al., 2001). In our data, genes coding for
452 thioglucosidases, like TGG1 and TGG2 are extremely down-regulated, and thus hydrolysis into

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453 toxic, antimicrobial components (Tab. S14). This fact also suggests the pathogen *P. brassicae*
454 inhibits plant defense response through suppression of the production of toxic antimicrobial
455 substances.

456

457 # Conclusion

458 An infection of brassicas with the biotrophic plant pathogen *Plasmodiophora brassicae,* often
459 leads to characteristic hypertrophied roots, the so called clubroots. Amongst other physiological
460 changes clubroot infection leads to an altered biosynthesis of cell wall components. Genes
461 coding for proteins involved in cellulose, hemicellulose, pectin, callose and lignin biosynthesis
462 like CesAs (cellulose synthase), Csls (cellulose synthase -like), and CalS/GSLs (callose
463 synthase) were down-regulated in infected plants. Also genes involved in pectin synthesis like
464 Rhm and Qua1, or in the initial steps of lignin biosynthesis (PAL, C4H, 4CL) were down-
465 regulated. Clubroot infection also leads to degradation and modification of existing wall
466 components what results in decreased wall rigidity. Transcripts belonging to
467 degrading/modifying enzymes, e.g. of pectins (PGs, PLs, PMEs, PAEs), cellulose through
468 cellulases with hydrolase activity, or callose through degradation and deposition were up-
469 regulated in clubroots. Gene expression for enzymes with degradation inhibiting activity were
470 down-regulated in infected tissue. Clubroot infection also leads to massive host cell expansion,
471 what we also see in transcriptomic data. Genes coding for XTHs and expansins, which allow
472 cell wall loosening were up-regulated. Also for WAK/L genes, which are involved in cell
473 elongation and defense response, gene expression differed. Elongation processes were up-
474 regulated and defense response was down-regulated in clubroots. Genes involved in plant
475 defense mechanisms leading to reinforcement of cell walls (e.g. callose synthesis, lignification)
476 were down-regulated in clubroots, whereas deposition of callose was up-regulated. All these
477 processes concur with observations during clubroot development, and the typical clubroot
478 phenotype is a result of efficient strategies of *P. brassicae* to influence host biology. How and
479 via which mechanisms this takes place remains to be answered with more targeted studies in
480 the future.

481

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485

486

487 **Author Contribution and References**

488 JB and SN designed the research, SC performed bioinformatic and statistic analysis, JB and SC
489 interpreted data; JB, SC and SN wrote the manuscript; All authors read and approved the final
490 manuscript.

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729 **Figure legends**

730

731 **Figure 1: Clubroot disease in Brassica species.** A: Typical clubroot symptoms caused by the
732 soil-borne pathogen *Plasmodiophora brassicae*. Above ground growth of diseased plants is
733 impaired. The dashed line indicates the soil surface. *Brassicae* spp. with and without (*)
734 clubroot symptoms. (I), (II) *Brassica rapa* subsp. *perkinensis* (Chinese cabbage); (III) *Brassica*
735 *oleracea* var. *gongylodes* (kohlrabi); (IV) *Brassica oleracea* var. *capitata* (cabbage); (V)
736 *Brassica oleracea* var. *italica* (broccoli). B: Section through a healthy *B. rapa* root. Starch
737 granules are indicated with arrowheads. C: Section through a root gall of infected *B. rapa*.
738 *Plasmodiophora brassicae* plasmodia are marked with stars and resting spores with triangles.
739 Scale bar: 30 µm.

740

741 **Figure 2: Synthesis of plant cell wall components (A) and modulation of cell wall**
742 **components and related plant response (B).** (A): Cellulose and callose synthesis are mediated
743 through cellulose synthase (CeSA and callose synthase (CalS) in the plasma membrane. UDP-
744 glucose serves as substrate for the synthesis. Sucrose synthase breaks down sucrose and
745 generates UDP-glucose and fructose. Pectins and hemicelluloses are synthesized in the Golgi
746 apparatus. Native pectins and hemicelluloses with sufficient length are transported by vesicles,
747 secreted to the apoplast, and enzymatically incorporated into the cell wall. During infection
748 with the biotrophic clubroot pathogen *Plasmodiophora brassicae,* genes involved in the
749 synthesis of cellulose, hemicelluloses, pectins and callose are down-regulated. Genes coding
750 for sucrose synthase are up-regulated in clubroots. Up- and down-regulated processes in
751 clubroots is indicated with orange arrows. (B): Cell wall degrading and modulating enzymes
752 and their target components. Cellulases modulate cellulose fibrils, XTHs cleave and re-anchor
753 xyloglucan chains (hemicellulose), expansins disrupt linkages between hemicellulose and
754 cellulose, and pectinases cleave pectins. Depolymerized pectins (oligoglacturonases, OGs) are
755 bound and recognized by wall associated kinase/-like (WAK/L) and can trigger further plant
756 response cascades. Pectinase-inhibitors (PMEIs, PGIPs) inhibit PME and PG activity. During
757 infection with the biotrophic clubroot pathogen *Plasmodiophora brassicae,* genes like
758 cellulases, pectinases (cell wall component degradation) and expansins and XTHs (cell
759 expansion) are up-regulated in infected tissue. Enzyme inhibitors like PMEI or PGIP are down-
760 regulated. Genes coding for WAKs (involved in cell elongation) are up-regulated and for
761 WAKLs (stress response) are down-regulated in clubroots. Up- and down-regulated processes
762 in clubroots is indicated with orange arrows.

763