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Physcomitrella patens MAX2 characterization

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Physcomitrella patens MAX2 characterization suggests an ancient role for this F-box protein in photomorphogenesis rather than strigolactone signaling.

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Summary (<200 words)

- Strigolactones are key hormonal regulators of flowering plant development and are widely distributed amongst streptophytes. In *Arabidopsis*, strigolactones signal via the F-box protein MORE AXILLARY GROWTH2 (MAX2), affecting multiple aspects of development including shoot branching, root architecture and drought tolerance. Previous characterization of a *Physcomitrella patens* moss mutant with defective

strigolactone synthesis supports an ancient role for strigolactones in land plants, but the origin and evolution of signaling pathway components is unknown.

- Here we investigate the function of a moss homolog of MAX2, PpMAX2, and characterize its role in strigolactone signaling pathway evolution by genetic analysis.
- We report that the moss *Ppmax2* mutant shows very distinct phenotypes from the moss SL-deficient mutant. In addition, the *Ppmax2* mutant remains sensitive to strigolactones, showing a clear transcriptional strigolactone response in dark conditions, and the response to red light is also altered. These data suggest divergent evolutionary trajectories for strigolactone signaling pathway evolution in mosses and vascular plants.
- In *P. patens*, the primary roles for MAX2 are in photomorphogenesis and moss early development rather than in strigolactone response, which may require other, still unidentified, factors.

Key words: Bryophyte, Moss, Hormone signaling, Strigolactone, Photomorphogenesis, F-box protein.

Introduction

Strigolactones (SLs) are plant hormones that were first identified as root-exudate products, exogenously indicating the vicinity of a host plant to parasitic plants such as *Striga* (Cook et al. 1966) and Arbuscular Mycorrhizal (AM) fungi (Akiyama et al. 2005). Roles for SLs in a range of endogenous developmental processes including shoot branching and root architecture were more recently described (Waldie et al. 2014; Lopez-Obando et al. 2015). SLs are present in most land plants (Xie et al. 2010) and the charophyte algal sister lineage to land plants

(Delaux et al. 2012), but signaling pathways are expanded in land plants relative to charophytes (Bowman et al. 2017). Therefore, SLs are key candidate facilitators for plant terrestrialization 480 million years (MY) ago (Bowman et al. 2017).

SL synthesis and signaling pathways have well characterized roles in branching in seed plants such as pea, *Arabidopsis*, *Petunia* and rice (Al-Babili and Bouwmeester 2015; Waters et al. 2017). Genes cloned from SL-deficient mutants have identified synthesis steps requiring a carotenoid isomerase DWARF27 (D27), two CAROTENOID CLEAVAGE DIOXYGENASES (CCD7 and CCD8), at least one Cytochrome P450 MORE AXILLARY GROWTH 1 (MAX1) (Al-Babili and Bouwmeester 2015), and the oxidoreductase-like enzyme LATERAL BRANCHING OXIDOREDUCTASE (LBO) (Brewer et al. 2016). In parallel, the study of SL-insensitive mutants has implicated several gene families in SL signaling. The first step of SL signaling is hormone perception, and this requires an α/β hydrolase enzyme, DECREASED APICAL DOMINANCE2/DWARF14/RAMOSUS3 (DAD2/D14/RMS3), that has been shown to interact with and cleave SL molecules *in vitro* (Hamiaux et al. 2012; Nakamura et al. 2013; de Saint Germain et al. 2016; Yao et al. 2016). *Petunia* DAD2 and rice D14 have been shown to interact in the presence of SLs with the F-box proteins MORE AXILLARY GROWTH 2A (PhMAX2A) and DWARF3 (D3), which are orthologous to *Arabidopsis* MAX2 (Hamiaux et al. 2012; Jiang et al. 2013; Zhou et al. 2013; Zhao et al. 2014). The current model for SL signaling mostly builds on studies of shoot branching in angiosperms, proposing that SL perception by D14/AtD14 induces the recognition of specific target proteins by an $SCF^{D3/MAX2}$ complex. This process leads to ubiquitination and proteasome-mediated degradation of targets in a similar process to processes described for other plant hormones including gibberellins (Lopez-Obando et al. 2015; Waters et al. 2017). Whilst roles for MAX2 in SL signaling were first described around 15 years ago (Stirnberg et al. 2002; Johnson et al. 2006; Stirnberg et al. 2007; Shen et al. 2012), the identification of DWARF53 (D53)/SUPPRESSOR OF MAX2-LIKE (SMXL) proteins as putative targets of the $SCF^{D3/MAX2}$ complex is more recent (Jiang et al. 2013; Stanga et al. 2013; Zhou et al. 2013; Soundappan et al. 2015; Wang et al. 2015).

Arabidopsis max2 mutants were also isolated in early genetic screens for delayed dark-induced senescence (Woo et al. 2001), and light hyposensitivity (Shen et al. 2007). Whereas the involvement of SLs in leaf senescence has been confirmed (Yamada et al. 2014; Ueda and Kusaba 2015), the photomorphogenesis phenotype of *max2* mutants appears independent of the SL pathway (Shen et al 2012). Furthermore, a requirement for MAX2 in other butenolide

signaling pathways was demonstrated by the isolation of *max2* mutants in a genetic screen for insensitivity to smoke-derived karrikins (Nelson et al. 2011; Waters et al. 2012). Karrikins induce *Arabidopsis* seed germination and affect seedling photomorphogenesis through a similar but distinct signaling pathway to SLs (Scaffidi et al. 2014; Waters et al. 2014), and an α/β hydrolase protein closely related to D14, KARRIKIN INSENSITIVE 2 (KAI2), is required for the response to karrikins (Waters et al. 2012). Whilst karrikins have not been detected in plants, KAI2 is the presumed receptor of an unknown plant-produced KAI2-ligand (KL) (Scaffidi et al. 2013; Waters and Smith 2013; Conn and Nelson 2015). Thus, the MAX2 F-box protein is involved in several signaling pathways apart from strigolactone signaling.

Although several components of the strigolactone synthesis and signaling pathways are shared amongst land plants, their roles in early diverging land plant lineages and contribution to plant evolution are unknown (Bowman et al. 2017). We addressed this evolutionary question using the moss *Physcomitrella patens* (*P. patens*) as a model representing an ancient divergence in land plant evolution. Whilst *CCD7* and *CCD8* orthologues are found in the *P. patens* genome, a true orthologue of *MAX1* is absent (Delaux et al. 2012). We previously generated *Ppccd8* SL-deficient mutants and demonstrated SL-functions in repressing radial plant growth and gametophore branching (Proust et al. 2011; Hoffmann et al. 2014; Coudert et al. 2015). Consideration of signaling pathways has revealed no true orthologue of the *D14* receptor gene, but there are 13 *PpKAI2-LIKE* genes that are closer to the *KAI2* α/β hydrolase clade in *P. patens* (Delaux et al. 2012; Lopez-Obando et al. 2016a). Phylogenetic analyses have also identified a single putative homologue for the F-box protein gene *MAX2* (Delaux et al. 2012) and three to four *PpSMXL* genes (Zhou et al. 2013).

Here we wished to explore SL signaling pathway evolution, and we focused on the role of the *P. patens MAX2* gene, testing whether *PpMAX2* is involved in the SL response. We also generated *PpMAX2* KO mutants and characterized their response to SL and red light at the phenotypic and molecular level. Our data indicate that similarly to MAX2 from *Arabidopsis*, *PpMAX2* is involved in photomorphogenesis. However, *PpMAX2* is probably not involved in generating a SL response.

Materials and Methods

P. patens **growth conditions**

144 The Gransden wild-type strain of *P. patens* was used and grown as previously described in a
145 culture room at 24°C (day)/22°C (night) with a light regime of 16 h light/8 h darkness and a
146 quantum irradiance of 80 µE m^{-2} s^{-1} (Proust et al. 2011; Lopez-Obando et al. 2016b). For
147 phenotypic analysis, fragmented protonemal tissue was grown for 7 days on PP-NH_4 medium
148 (=PP-NO_3 medium supplemented with 2.7 mM NH_4-tartrate) then transferred to PP-NO_3
149 medium (Ashton et al. 1979; Hoffmann et al. 2014). Sporogenesis was induced in Magenta
150 vessels in which 21/28-day-old plants were grown on soil plugs (or PP-NO_3 medium) for 10
151 days as above and then transferred to a growth chamber at 15°C with 8 h of light per day and
152 a quantum irradiance of 15 µE m^{-2} s^{-1} and rinsed once a week with sterile tap water till
153 capsule maturity (after 2 to 3 months). For red light experiments, plants were grown on PP-
154 NO_3 medium, at 24°C with a continuous red-light regime of 46 µE m^{-2} s^{-1}.
155
156 **Generation of *Ppmax 2-1, Ppmax2-2* and *Ppccd8-Ppmax2* mutants**
157 Moss protoplasts were obtained and transformed as described previously (Trouiller et al.
158 2006). For the *Ppmax2-1* mutant, a 735 bp *PpMAX2* genomic 3' CDS flanking sequence
159 fragment was cloned in the pBHRF vector (Thelander et al. 2007), digested with *Nar*I and
160 *Hpa*I. Next, an 886 bp *PpMAX2* genomic 5' CDS flanking sequence fragment was inserted
161 into *Avr*II/*Xho*I sites of the pBHRF vector carrying the 3' CDS flanking sequence (PpMAX2-
162 KO1 construct). For the *Ppmax2-2* mutant, a 1170 bp 5' CDS flanking sequence fragment and
163 a 1170 bp 3' CDS flanking sequence fragment were amplified and subcloned into pJET1.2
164 vector (Fermentas) with a Geneticin/G418 resistance cassette from pMBL11a plasmid
165 (Knight et al. 2002) (PpMAX2-KO2 construct). WT protoplasts were transformed with the
166 PpMAX2-KO1 or the PpMAX2-KO2 constructs, and transformants were selected on 30 mg l^{-}
167 ^{1} Hygromycin B or 50 mg l^{-1} G418, respectively. For the *Ppccd8-Ppmax2* double mutant,
168 protoplasts from the single *Ppccd8* mutant were transformed with a construct carrying the
169 same flanking sequences as PpMAX2-KO2, subcloned into the pJET1.2 vector, with a
170 Hygromycin resistance cassette from pMBLH8a (Knight et al. 2002). Transformants were
171 selected on 30 mg l^{-1} Hygromycin B. Stable transformants of the *PpMAX2* gene were
172 confirmed by PCR using specific primers (Fig. S1 and Table S1).
173
174 **Protoplast assays**
175 Protoplasts were isolated as described in (Trouiller et al. 2006), counted, and kept overnight
176 in the dark at 24°C, in liquid 8.5 % mannitol PP-NH_4. The next day, drops of 750 protoplasts
177 gently mixed with 0.7% top agar (v/v) were transferred on 8.5 % mannitol PP-NH_4 plates,

178 with various (0 to 3 μM) concentrations of (±)-GR24 for 5 days, prior to transfer onto plates
179 without mannitol (but with (±)-GR24).
180
181 **Molecular cloning and subcellular protein localization**
182 *Generating the PPpMAX2:GUS lines*
183 The *ZmUbi-1* promoter was eliminated from the pMP1300 vector
184 [http://labs.biology.ucsd.edu/estelle/Moss_files/pMP1300-K108N+Ubi-GW-GUS.gb] by
185 PCR amplification using primers Ubi-pr and Ubi-exp (Table S1) and the plasmid backbone
186 was self-ligated and renamed pMP1301. A 1961 bp promoter region for *PpMAX2* was
187 amplified from *P. patens* gDNA using primers PPpMAX2_F and PPpMAX2_R (Table S1).
188 The product was purified and cloned into the vector pCR®8/GW/TOPO® (Life Technologies®,
189 USA-CA). An LR-clonase reaction between the pMP1301 and pCR8::*PPpMAX2* plasmids
190 yielded *PPpMAX2*:GUS, which was used to transform WT *P. patens*. A stable G418 resistant
191 line was used for subsequent histochemical analysis to determine GUS localisation.
192
193 *Generating the ZmUbi:gfp:PpMAX2 lines*
194 Single-stranded *P. patens* cDNA was used as template to amplify the *PpMAX2* coding
195 sequence using the PpMAX2_F and PpMAX2_R primer set (Table S1). The 2493 bp product
196 was cloned into the pCR®8/GW/TOPO®. pCR8::PpMAX2 was recombined with the
197 pMP1335 vector [http://labs.biology.ucsd.edu/estelle/Moss_files/ pK108N+Ubi-mGFP6-
198 GW.gb] to get pMP1335::*PpMAX2*. pMP1335::*PpMAX2* was linearised by *Sfi*I digestion and
199 transformed into WT *P. patens*. Stable G418 resistant lines were screened for insertion by
200 PCR using the GFP_F and PpMAX2_R primers (Table S1). For one of these positive
201 *GFP*:*PpMAX2* lines the localisation of the recombinant GFP:PpMAX2 was determined by
202 visualising protonemal tissue on a confocal microscope (Carl Zeiss Confocal LSM 780 Elyra
203 with SR- SIM superresolution plasform). For analysis, protonemal tissue was fixed in 4%
204 (v/v) formaldehyde for 10 min and then stained with a 0.0125% (w/v) Hoescht33342 solution.
205 Images were analysed by the ZEN 2012 (blue edition) software package (Carl Zeiss,
206 Germany).
207
208 ***Arabidopsis* complementation and phenotyping experiments**
209 Constructs in which the *PpMAX2* coding sequence was constitutively expressed alone or in a
210 GFP fusion were introduced into the *max2-3* (N592836) T-DNA insertion mutant. The
211 pUbi10 promoter, corresponding to the first 634 base pairs immediately upstream of the

212 ubiquitin-10 gene from Arabidopsis (At4g05320) was used to drive *PpMAX2* expression
213 (Grefen et al. 2010). Expression of the *PpMAX2* mRNA and/or fluorescence of the GFP were
214 checked in the corresponding transformed lines (Fig. S2). Results for two independent lines
215 carrying each *PpMAX2* construct are shown. Hypocotyl length under low fluence experiments
216 were carried out as previously described (de Saint Germain et al. 2016).
217
218 **RNA extraction and gene expression analyses**
219 Gene expression analyses were done by reverse-transcription quantitative PCR (RT-qPCR) as
220 previously described (Hoffmann et al. 2014; Lopez-Obando et al. 2016a), with primers listed
221 in Table S1.
222
223 **Statistical analyses**
224 For statistical analyses, ANOVA and Kruskal-Wallis tests were used (R Commander version
225 1.7-3).
226
227 Results

228 ***Physcomitrella patens* contains a single *MAX2* homologue**

229 The single *P. patens MAX2* homologue (Pp3c17_1180v3) was named *PpMAX2* (Delaux et al.
230 2012; Li et al. 2016). Phylogenetic analysis of full-length predicted MAX2 proteins indicated
231 that, in contrast to previously published phylogenies that used a higher number of EST and
232 full-length sequences, MAX2 from *P. patens*, *Marchantia polymorpha* and *Selaginella*
233 *moellendorffii* formed a separate clade to seed plant proteins (Fig. S3a) (Delaux et al. 2012;
234 Bythell-Douglas et al. 2017). Thus, the precise relationships between MAX2 homologues in
235 vascular plants and those in non-vascular plants remain ambiguous. Nevertheless, the lack of
236 any other close homologue in moss and the fact that *MAX2* is present as a single copy gene in
237 a large majority of plant genomes suggest that *PpMAX2* is likely orthologous to *AtMAX2*.
238 *PpMAX2* has no intron (Fig. S3b), and the predicted PpMAX2 protein is larger than vascular
239 plant MAX2 proteins, containing C terminal insertions (Fig. S3c). Alignment of several
240 predicted MAX2 protein sequences from vascular plants and bryophytes showed that
241 PpMAX2 has a conserved F-box domain and similar LRR repeats composition to AtMAX2,
242 with the exception that LRR13 is longer and consequently could not be modeled to existing F-
243 box structures in this region (Fig. S3c-d).
244

245 ***PpMAX2* is expressed in most cells, and PpMAX2 localizes to the nucleus**

246 To characterize the expression profile of *PpMAX2*, a 1961 bp promoter fragment was cloned
247 upstream of the GUS coding sequence and introduced into the neutral Pp108 locus of wild-
248 type (WT) moss plants by targeted insertion (Schaefer and Zryd 1997). Expression of the
249 GUS reporter was observed in protonemal filaments, but not at the very tips of caulonema
250 (Fig. 1a). Expression was also observed in gametophore axes and leaves (Fig. 1a-d), with
251 stronger staining in older leaves than in young leaves at the top of the gametophore (Fig.
252 1c,d). This pattern was corroborated by expression data from the *P. patens* eFP-Browser and
253 Genevestigator public databases (Hiss et al. 2014; Ortiz-Ramirez et al. 2016), that also
254 indicated strong expression in sporophytes (Fig. S4). To determine the sub-cellular
255 localization of PpMAX2, a GFP sequence was inserted in-frame and upstream of the
256 PpMAX2 protein coding sequence, and introduced into WT plants. In accordance with
257 knowledge of F-box protein function from flowering plants (Stirnberg et al. 2007), PpMAX2
258 localized to nuclei in protonemal cells (Fig. 1e-h).

259

260 ***Ppmax2* mutants are small plants with few but large gametophores, and show converse**
261 **phenotypes to *Ppccd8* mutants**

262 To determine the role of PpMAX2, *Ppmax2* mutants were engineered by targeted replacement
263 using two replacement strategies, and two independent knockout lines were obtained (Fig.
264 S1a,b). Whilst regeneration efficiencies were very low relative to WT plants (not shown),
265 both *Ppmax2-1* and *Ppmax2-2* mutants showed the same phenotype (Fig. 2a) with very few
266 protonema and rapid differentiation of large gametophores relative to WT plants (Fig. 2a).
267 *Ppmax2* mutants were small with limited growth after several weeks of culture (Fig. 2b,c).
268 When grown on soil plugs, plant diameter and the number of gametophores per plant were
269 considerably reduced (Fig. 2c-d) and no sporophytes were found. We also tested the effect of
270 the *Ppmax2* mutation on gametophore branch patterning (Coudert et al. 2015). Although the
271 size of the apical inhibition zone (the apical portion of gametophores devoid of branches) was
272 slightly smaller and the overall branch number was slightly higher in *Ppmax2-1* mutants than
273 in WT plants, the spacing between branches was similar in both genotypes (Fig. 2e, Fig S5).
274 These data suggest that *PpMAX2* plays a minor role in gametophore branching. If *PpMAX2*
275 has roles in moss SL signaling, we would expect that the phenotype of *Ppmax2* mutants
276 should resemble *Ppccd8* SL biosynthesis mutant phenotype, as in flowering plants (Gomez-
277 Roldan et al. 2008; Umehara et al. 2008). However, *Ppmax2* and *Ppccd8* appear to have

278 opposite phenotypes, as if *Ppmax2* displayed SL over-production or a constitutive SL
279 response (Fig. 2a,b,d).

280

281 ***Ppmax2* mutants can elicit a strigolactone response**

282 As SL molecules are very difficult to quantify, we used an indirect approach to determine
283 whether *Ppmax2* overproduces SL and quantified expression of *PpCCD7*, a SL-responsive
284 gene whose transcript levels decrease following (±)-GR24 application (Proust et al. 2011).
285 We used *Ppccd8* mutant plants for these experiments as the SL response is easier to observe
286 in mutants than in WT plants (Hoffmann et al. 2014), and plants were transferred onto media
287 containing no exudate, 1 µM (±)-GR24, WT, *Ppccd8* or *Ppmax2-1* exudate. *PpCCD7*
288 transcript levels were assayed 6 h after transfer (Fig. 3). Transfer of plants onto medium
289 containing *Ppccd8* exudate led to *PpCCD7* transcript levels similar to those observed
290 following transfer onto fresh medium. However, transfer onto medium containing *Ppmax2-1*
291 exudate led to a significant decrease of *PpCCD7* transcript level, as was observed following
292 transfer onto media containing (±)-GR24 or WT exudate (Fig. 3). Thus *Ppmax2-1* exudate
293 affects *PpCCD7* transcript levels in a similar way to WT exudate, and *Ppmax2-1* is likely to
294 produce SL at similar levels to WT plants.

295

296 ***Ppmax2* mutants show growth responses to (±)-GR24 application**

297 The response of *Ppmax2* mutants to exogenously applied (±)-GR24 was tested and compared
298 to the response of *Ppccd8* mutants to identify any roles for PpMAX2 in SL signaling. These
299 experiments were carried out using dark-grown caulonema where differences in growth are
300 most pronounced (Hoffmann et al. 2014), and plants were grown vertically so that caulonema
301 extending with a negative gravitropism on the medium could be directly measured. Under
302 these conditions both *Ppmax2-1* and *Ppccd8* mutant caulonema showed significant and dose-
303 dependent growth suppression (Fig. 4a). The relative decrease in caulonema length was
304 greater in the *Ppmax2-1* mutant than in *Ppccd8* in all tested conditions (Fig. 4a). We also
305 assayed SL responsiveness using a protoplast regeneration assay, and found that fewer plants
306 regenerated in WT and *Ppccd8* and *Ppmax2* mutant plants following (±)-GR24 application,
307 with the response being dose-dependent (Fig. 4b). Thus, *Ppmax2* mutants can respond to SL
308 application, and the response is pronounced in caulonema when mutants are grown in the
309 dark, or in protoplasts regenerating in the light.

310

311 ***Ppmax2* mutants show transcriptional responses to (±)-GR24 application**

312 *Ppmax2* responses to SL were further analyzed using SL-responsive genes as molecular
313 markers. The *PpCCD7* transcript level was very low in *Ppmax2-1* mutants relative to levels in
314 *Ppccd8* mutants and WT plants, and in contrast to a significant response observed in WT and
315 *Ppccd8,* no significant decrease was noted in *Ppmax2-1* mutants 6 h after transfer on medium
316 with (±)-GR24, (Fig. 4c). We also measured transcript abundance of the *PpKUF1LA* gene
317 (Pp3c2_34130v3.1), a moss homologue of *Arabidopsis KAR-UP F-BOX1* (*KUF1*). *KUF1*
318 transcript levels are sensitive to (±)-GR24 application in *Arabidopsis* SL biosynthesis mutants
319 (Nelson et al. 2011; Waters et al. 2012; Stanga et al. 2016). *PpKUF1LA* (Pp3c2_34130v3.1)
320 transcript levels increased 6 h after transfer on medium containing (±)-GR24 in both light-
321 grown WT and *Ppccd8* mutants, but no response was detected in light-grown *Ppmax2-1*
322 mutants (Fig. S6a). As the bioassay suggested a *Ppmax2-1* response to SL in the dark (Fig.
323 4a), we tested gene expression in dark grown plants. In contrast to WT and *Ppccd8* mutant
324 plants, no decrease of the *PpCCD7* transcript level was observed in *Ppmax2* mutants
325 following transfer on (±)-GR24 (Fig. S6b). However, in dark-grown *Ppmax2-1* plants,
326 transcript levels of *PpKUF1LA* significantly increased following transfer on (±)-GR24 as in
327 WT and *Ppccd8* mutant plants (Fig. 4d). Thus *Ppmax2* mutants remain responsive to
328 exogenously-applied SL.

329

330 ***PpMAX2* expression is light responsive, and *Ppmax2* has impaired light responses**

331 To further investigate roles for *PpMAX2* in light-regulated development, WT tissues were
332 grown in the light for 7 days and then placed in the dark for 5 days prior to transfer into red
333 light for increasing lengths of time. *PpMAX2* transcript levels were higher in the dark than in
334 the light (Fig. 5a). One hour of red light treatment led to a significant decrease in *PpMAX2*
335 transcript levels, and a 3-hour treatment resulted in a minimal expression level that was
336 comparable to *PpMAX2* expression levels in white light (Fig. 5a), thus *PpMAX2* expression is
337 light regulated. In white light, gametophores with the same number of leaves as WT, *Ppccd8*
338 or *Ppmax2-2* mutant plants were taller in *Ppmax2-2* mutants (Fig. 5b), showing an etiolation
339 phenotype associated with light regulated development in other plants. To investigate a
340 potential role for *PpMAX2* in photomorphogenesis, *Ppmax2-1* mutants were grown under
341 continuous red light for 25 days. A strong etiolation phenotype was observed in *Ppmax2-1*
342 mutant gametophores but not in WT or *Ppccd8* (Fig. 5c). We analyzed the transcript levels of

genetic markers for light response in WT versus *Ppmax2-1* mutant tissues. *Ppmax2-1* mutant and WT plants were first grown in white light for 2 weeks and then transferred into the dark for 4 days prior to exposure to red light for increasing time periods (0.5h to 24h). After red light treatment, transcript levels of both *ELONGATED HYPOCOTYL 5a* (*PpHY5a*) and *NADPH-PROTOCHLOROPHYLLIDE OXIDOREDUCTASE 1* (*PpPOR1*) were measured by RT-qPCR (Fig. 5d,e). The transcript levels of *PpHY5a* showed a transient and rapid increase with red light exposure in WT whilst remaining almost unchanged in *Ppmax2-1*. *PpPOR1* transcript levels also increased with red light exposure in WT but remained lower in *Ppmax2-1*. The *Ppmax2* mutants thus have an impaired response to red light.

Ppmax2 is epistatic to _Ppccd8_

To examine the genetic interaction between *PpMAX2* and *PpCCD8*, *Ppmax2* mutants were engineered in the *Ppccd8* mutant background (Fig. S1c). *Ppccd8-Ppmax2* double mutants had a phenotype similar to that of *Ppmax2* mutants, with no additive effects on plant extension or gametophore development, indicating that the *Ppmax2* mutation can override the effect of the *Ppccd8* mutation (Fig. 6a,b). Whilst up-regulated *PpCCD7* transcript levels are a genetic marker of *Ppccd8* mutants, *PpCCD7* expression was down-regulated in both *Ppmax2-1* and *Ppccd8-Ppmax2* double mutants (Fig. 6c), further suggesting that the *Ppmax2* mutation is epistatic to the *Ppccd8* (Fig. 6a,b).

PpMAX2 cannot complement _Atmax2_ mutant phenotypes

The data above suggest that roles for MAX2 in SL signaling are not conserved between *P. patens* and *Arabidopsis*. To test this hypothesis, we heterologously expressed *PpMAX2* in the *Atmax2-3* mutant background, and used *AtMAX2* as a control (Fig. 7, FigS2). Whilst *AtMAX2* expression was able to restore WT plant phenotypes, *PpMAX2* expression failed to complement the reduced height, higher branching and elongated hypocotyl under low fluence mutant phenotypes of *Atmax2-3* (Fig. 7). Some partial complementation of the branching phenotype was observed in the lines where the *PpMAX2* gene was fused to the GFP, with intermediate branching between WT and *Atmax2-3* (Fig. 7c). However, as these lines are smaller in size than the *Atmax2-3* mutant (Fig. 7a), one cannot conclude that these were complemented lines. Thus *PpMAX2* and *AtMAX2* are not functionally equivalent.

375 Discussion

376 Phylogenetic studies have suggested that SL biosynthesis and signaling pathways are
377 conserved amongst land plants (Proust et al. 2011; Delaux et al. 2012; Waters et al. 2012;
378 Bowman et al. 2017). SLs or SL-like compounds are found in bryophytes and in the moss, *P.*
379 *patens*, both the PpCCD7 and PpCCD8 proteins have been shown to have *in vitro* enzymatic
380 activities that are conserved with seed plants, indicating probable conservation of at least the
381 early steps in SL biosynthesis (Decker et al. 2017). Homologues of key genes of the SL
382 signaling pathway are found in the *P. patens* genome, with one *PpMAX2*, 13 *PpKAI2-LIKE*
383 and four *PpSMXL* genes. Whilst it is likely that some of the KAI2 proteins may function as
384 SL receptors in moss (Lopez-Obando et al. 2016a), as yet no functional studies demonstrate
385 their involvement in SL perception. This study focused on the moss *PpMAX2* gene and our
386 results indicate that roles in photomorphogenesis are conserved with *Arabidopsis* MAX2, but
387 that a role of PpMAX2 in SL signaling is unlikely.

388

389 **SL signaling pathway in moss is distinct from flowering plants, and does not require the**
390 **PpMAX2 F-box protein**

391 The *Ppmax2* phenotype and the ability of the mutant to respond to SL are evidence that
392 PpMAX2 is not necessary for SL signaling. Gametophore branching (Coudert et al. 2015) and
393 plant spread phenotypes are different between the *Ppccd8* and *Ppmax2* mutants. These results
394 contrast with mutant phenotypes in seed plants, where shoot branching and plant height are
395 comparable in *ccd8* and *max2* mutants (Gomez-Roldan et al. 2008; Umehara et al. 2008). In
396 *Arabidopsis*, the *max2* mutation is considerably more pleiotropic in comparison to the *ccd8*
397 (*max4*) mutation. SL-independent seed germination and photomorphogenesis phenotypes are
398 observed in *Atmax2* mutants (Nelson et al. 2011; Shen et al. 2012). As both SL and the
399 unidentified KAI2-Ligand (KL) signal through AtMAX2, the mutant combines the effect of
400 alteration of several pathways. It is possible that in moss the *Ppmax2* mutation is also highly
401 pleiotropic and that the strong effect of the *Ppmax2* mutation masks or overrides the *Ppccd8*
402 phenotype. This hypothesis is supported by the *Ppccd8-Ppmax2* double mutant phenotype
403 that resembles the *Ppmax2* phenotype.

404 Several bioassays were used to test the SL response of the *Ppmax2* mutant, and the *Ppmax2*
405 mutant is sensitive to (±)-GR24 applications under protoplast regeneration and early growth
406 in light conditions, as well as during caulonemal growth in the dark. Furthermore, a

407 transcriptional response of SL-responsive genes in the *Ppmax2* mutant is observed in dark
408 conditions. We observed that the scale of the *Ppmax2* response to (±)-GR24 was variable
409 compared to that of WT or *Ppccd8* mutants (Fig. 4). This may be related to the use of racemic
410 (±)-GR24 that could induce SL-independent effects (Scaffidi et al. 2014), not yet
411 characterized in moss. The fact that PpMAX2 expression does not restore the *Arabidopsis*
412 *max2* phenotypes also argues against a role in SL response, although the moss PpMAX2 F-
413 box protein may not be able to recognize *Arabidopsis* protein interaction partners in
414 transformed lines due to differences in C-terminus protein structure (Fig. S3,c).

415 Our conclusion that PpMAX2 is not crucial for SL signaling in moss leads us to hypothesize
416 that other factors (e.g. F-box proteins) may be required. Interestingly, MAX2-independent SL
417 responses have previously been hypothesized for roots of seed plants (Ruyter-Spira et al.
418 2011; Shinohara et al. 2013; Walton et al. 2016) and high doses of (±)-GR24 (5-10 μM) can
419 induce a response in *Arabidopsis max2* mutants (Ruyter-Spira et al. 2011). Furthermore,
420 MAX2-independent promotion of stromule formation can be induced by (±)-GR24 (Vismans
421 and van der Meer 2016). An unknown factor involved in SL signaling could thus be
422 conserved between moss and vascular plants and able to signal with more subtle effects than
423 the MAX2 pathway. SL signaling in moss could also be F-box protein independent,
424 implicating different downstream mechanisms to those so far described in vascular plants in
425 signaling. Investigation of the roles of *PpSMXL* genes, and putative degradation of PpSMXL
426 proteins should clarify this point in the future.

427

428 **Do *Ppmax2* and *Ppccd8* mutants really have opposite phenotypes?**

429 The response to SL of the *Ppmax2* mutant was difficult to pinpoint because *Ppmax2* mutants
430 have a converse phenotype to *Ppccd8* mutants. Whilst *Ppmax2* mutant plants are small and
431 have few protonemal filaments, *Ppccd8* plants produce many protonemata and spread across
432 the substrate. We previously showed that whilst WT plants cease protonemal spread in
433 response to near neighbors, *Ppccd8* mutants are insensitive to neighbors in Petri cultures
434 (Proust et al. 2011). This phenomenon leads to small plant size as in the *Ppmax2* mutants and
435 WT plants grown on high (non-physiological) doses of (±)-GR24 are also small with
436 comparable size to *Ppmax2* plants (Fig. S7). Another line of evidence supporting the
437 interpretation that *Ppmax2* and *Ppccd8* mutant phenotypes are converse is the transcript level
438 of several SL-responsive genes, conversely affected in *Ppccd8* and *Ppmax2* mutants. For

439 instance, *PpCCD7* transcript levels are very low in *Ppmax2* but much higher in *Ppccd8*
440 mutants (Fig. 4c).

441 If the phenotypes of *Ppccd8* and *Ppmax2* mutants are converse, *Ppmax2* plants may over-
442 produce and/or over-accumulate SLs. This hypothesis was tested indirectly by monitoring the
443 *Ppccd8* mutant response to *Ppmax2* exudate versus *Ppccd8* or WT exudates or (±)-GR24
444 treatment (Fig. 3), and the results suggest that *Ppmax2* does not over-produce SLs, but
445 verification by SL quantification is required, and these assays are challenging in moss.
446 Alternatively, *Ppmax2* mutants could phenocopy a constitutive SL response.

447 As PpMAX2 is an F-box protein, putatively involved in degradation processes by the
448 proteasome system, PpMAX2 could target activators of SL signaling for degradation, and
449 such activators so far remain unidentified. SMXL proteins are known targets for degradation
450 in seed plant SL signaling pathways, and SMXLs are considered as repressors of this pathway
451 (Soundappan et al. 2015; Wang et al. 2015). Interestingly, the converse phenotypes of
452 *Ppmax2* and *Ppccd8* mutants did not hold for gametophore branching, as *Ppmax2*
453 gametophores did not lack branches as in a pea *CCD8* overexpressor line (*PpRMS1OE*)
454 (Coudert et al. 2015). PpMAX2 may function in protonema and early gametophore
455 development, but not in later development (Fig. 8).

456 The low levels of *PpCCD7* expression in *Ppmax2* in comparison to WT suggest that
457 PpMAX2 and SL are not completely independent. However, this could be an indirect effect of
458 reduced gametophore production in the mutant (Fig. 2d) as the highest *PpCCD7* transcript
459 levels were observed at the base of the gametophore (Proust et al. 2011). There may also be
460 indirect feedback control on transcript levels. In vascular plants, environmental conditions (N,
461 P, drought) or endogenous factors as auxin control the expression levels of SL biosynthesis
462 genes (Al-Babili and Bouwmeester 2015; Ligerot et al. 2017). It would be interesting to
463 quantify auxin levels in both *Ppmax2* and *Ppccd8* mutants to test whether differences in IAA
464 levels translate into differences in *PpCCD7* transcript levels. Further experiments are needed
465 to have a clear understanding of the moss SL signaling pathway. In particular, biochemistry to
466 test protein interactions and quantification of the levels of other hormones should be very
467 informative.
468

469 **The role of MAX2 in light response is similar between moss and seed plants**

470 The shoot elongation phenotype of the *Ppmax2* mutant under red light and its misregulation
471 of light responsive genes support the notion that *PpMAX2* plays a role in light responses (Fig.
472 5), as does its flowering plant homologue (Shen et al. 2007). The paucity of caulonemal
473 filaments in *Ppmax2* may also be related to a defective light response as a similar phenotype
474 was observed in the light sensing-defective *P. patens Δhy5ab* and *pubs-hy2* double mutants
475 (Yamawaki et al. 2011; Chen et al. 2012). In our experiments, tissue used for RNA extraction
476 included a mix of protonemata and gametophores, and the ratio of different tissue types may
477 be different in mutants versus WT plants given their distinct phenotypes. Whilst we interpret
478 the light responsive gene expression data with caution, our results suggest that the ancestral
479 role of MAX2 may be to promote photomorphogenesis. Despite this likely shared role with
480 AtMAX2, PpMAX2 cannot complement the *Atmax2* mutant hypocotyl phenotype under low
481 fluence light (Fig. 7d), potentially because *Arabidopsis* MAX2 and moss PpMAX2 protein
482 partners may not recognize one another. As with the shade avoidance response of vascular
483 plants it is possible that PpMAX2 helps plants to grow in an ideal amount of light. In this
484 instance, PpMAX2 could allow plants to respond to low light, delaying gametophore growth
485 and investing energy in spreading protonemal tissues to find light patches. This regulation
486 could also require HY5, given the similar phenotypes of the mutants (see above) and the
487 misregulation of *HY5a* transcript levels in the *Ppmax2* mutant.

488

489 **An ancestral role of MAX2 in moss development**

490 Our data and our model for roles for MAX2 in land plants (Fig. 8) open the question of an
491 evolutionary benefit to seed plants in recruiting this F-box protein to SL signaling. We
492 propose that combining the ability of MAX2 to regulate the levels of downstream proteins
493 (e.g. SMXL proteins) would have added a level of fine (endogenous) regulation to
494 photomorphogenesis or aspects of development already under the control of this F-box
495 protein in early land plants. Further studies in other land plants including gymnosperms,
496 lycophytes and other bryophytes will answer this question.

497 The expression of the *PpMAX2* gene during all stages of moss development is in agreement
498 with the putative function of PpMAX2 as a component of an SCF complex regulating the
499 homeostasis of multiple targets. Phenotypes of *Ppmax2* mutants and the *Ppccd8-Ppmax2*
500 double mutant indicate an early and simultaneous role in repressing gametophore/bud
501 differentiation and stimulating the chloronema to caulonema transition. Thus PpMAX2 could
502 act conversely to SLs which repress plant spread (Proust et al. 2011). Interestingly, in moss,

503 auxin has been shown to regulate the chloronema to caulonema transition (Ashton et al. 1979;
504 Prigge et al. 2010; Jang and Dolan 2011), while cytokinins induce bud differentiation (von
505 Schwartzenberg et al. 2007). It would thus be interesting to investigate both the auxin and
506 cytokinin status of the *Ppmax2* mutant. Involvement of all three hormonal pathways in moss
507 gametophore branching has been recently addressed (Coudert et al. 2015), and this study
508 suggests that auxin, cytokinin and SL signaling may interact, as in vascular plants.

509 In seed plants, MAX2 has been linked to signaling by a still unknown KL compound "which
510 interacts at some level with auxin and light signaling to regulate growth and development"
511 (Waters and Smith 2013; Conn and Nelson 2015). As the receptor KAI2 is ancestral, this
512 pathway may be present in bryophytes. It could then be argued that the *Ppmax2* phenotype is
513 the consequence of disturbing this second signaling pathway (Fig. 8). Given this scenario, KL
514 signaling could interfere with or mask SL signaling, because the phenotype of the *Ppccd8-*
515 *Ppmax2* double mutant is closer to that of *Ppmax2*. It has not yet been possible to test this
516 hypothesis as moss does not seem to respond to karrikins (Hoffmann et al. 2014) and the
517 nature of KL compound is still elusive. The study of interactions of PpMAX2 with some of
518 the 13 PpKAI2-LIKE and/or the four PpSMXL putative targets found in moss genome
519 (Bennett and Leyser 2014; Lopez-Obando et al. 2016a) will be key to confirming the place of
520 PpMAX2 in these signaling events.

521

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527

528

529 **Author contributions**

530 CR, SB, ML-O, JK, RdV, PH, JH and YC designed the research. BH, LM, ML-O, YC, ASG,
531 PH, RdV and SB conducted experiments, SB, ML-O, PH, JH, YC and CR analyzed data and
532 wrote the article with contribution of all authors.

533
534

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Figure legends:

Fig. 1: Pattern of *Physcomitrella patens PpMAX2* gene expression and subcellular localization of the protein. (a-d): Pattern of *PpMAX2* gene expression by staining of a moss line expressing the GUS coding sequence under the control of *PpMAX2* gene promoter (inserted in Pp108 locus) (a) protonema cells, (b-d) gametophore leaves and stems; arrow in (c): rhizoids. scale bars: (a-b): 0.1 mm; (c): 1 mm (d): 0.5 mm. (e-h) Nuclear localization of PpMAX2 in a protonemal tip cell of a WT moss line transformed with a mGFP6::PpMAX2 translational fusion by homologous recombination. (e) Nucleus labeling with Hoescht33342. (f) GFP fluorescence (g) chloroplast autofluorescence (h) Merge of all 3 images (e-g), indicating co-localization of Hoescht33342 and GFP to the nucleus. Scale bar: 20 µm.

Fig. 2: *Physcomitrella patens Ppmax2* mutants are affected in development and show contrasting phenotype to the *Ppccd8* SL synthesis mutant. (a): Bright field photographs of 7 day-old (left), 13 day-old (middle) and 20 day-old plants (right). Scale = 500 µM (b): Comparison of *Ppmax2* mutants plant diameter to that of WT and *Ppccd8* mutant after 5 weeks (left, mean ± SE of 3 plates with 16 plants measured per plate) and 5 month (right, mean ± SE of 10 plants grown on soil plugs) growth in the light. Asterisks denote significant differences between WT and mutants based on a Kruskal–Wallis test ($P < 0.001$) (c) Pictures of 5 month-old WT and *Ppmax2-1* plants grown on soil plugs. (d): Comparison of *Ppmax2-1* mutant fitness to that of WT and *Ppccd8* mutant in 5 month-old plants, measuring gametophore number per plant (left) and sporangia number per plant (right). Data are means of 10 plants ± SE. Asterisks denote significant differences between the genotypes based on a Kruskal–Wallis test (**$P<0.01$; ***$P<0.001$) (e): *Ppmax2-1* gametophore branching pattern

567 compared to that of WT (left panel). Apical inhibition zone size (middle panel) was reduced
568 in *Ppmax2-1* (mean ± SD; bilateral t-test different from WT,*p < 0.05), while distance to
569 closest branch was similar (mean ± SD).

570

571 Fig. 3: The *Physcomitrella patens Ppmax2* mutant exudate tested on *PpCCD7* expression is
572 similar to WT

573 Three-week-old *Ppccd8* plants were transferred for 6h on medium with 0µM (±)-GR24 (Ctl),
574 or 1µM (±)-GR24, or on medium where the WT, or the different mutants had grown (and
575 exuded SLs) for 3 weeks noted as "exud". Data represent means of transcript levels of 3
576 biological repeats relative to *PpAPT* expression level, ± SE. Different letters indicate
577 significantly different results based on a post-hoc Kruskal–Wallis test (P < 0.05).

578

579 Fig. 4: The *Physcomitrella patens Ppmax2* mutant is sensitive to the synthetic SL (±)-GR24.
580 (a) Caulonema length measurements in the dark in *Ppmax2-1* mutant and *Ppccd8* SL
581 synthesis mutant, following application of increasing concentrations of (±)-GR24. Control
582 (Ctl): same amount of acetone. Asterisks denote significant differences between the control
583 and the treatment within the genotypes based on a Kruskal–Wallis test (P < 0.001). (b)
584 Protoplast regeneration tests. Asterisks denote significant differences between the control and
585 the treatment within the genotypes based on a Kruskal–Wallis test (P < 0.001). (c) Transcript
586 levels of the SL responsive gene *PpCCD7* relative to *PpAPT* and *PpACT3* transcript levels in
587 WT, *Ppccd8* and *Ppmax2-1* grown for 3 weeks in the light. (d) Transcript levels analysis of
588 the SL responsive gene *PpKUF1LA* relative to *PpAPT* and *PpACT3* transcript levels in WT,
589 *Ppccd8* and *Ppmax2-1* mutants, grown for two weeks in the light then one week in the dark
590 and transferred onto control medium (Ctl) or 3 µM of (±)-GR24. On the right, a close-up of
591 transcript levels in *Ppmax2-1* is shown. Different letters indicate significantly different results
592 between non-treated genotypes based on a Kruskal–Wallis test (P < 0.05). Asterisks denote
593 significant differences between treated and control plants within a genotype based on a *post-*
594 *hoc* Kruskal–Wallis test (P < 0.001). Data represent means of 3 biological repeats, relative to
595 mean (*PpAPT-PpACT3*) transcript level ± SE.

596

597 Fig. 5: The *Physcomitrella patens Ppmax2* mutant has impaired photomorphogenesis. (a)
598 Transcript levels of *PpMAX2* gene in WT, following growth in the dark (5 days) then in red

599 light for increasing lengths of time (0.5h to 24h). Controls: growth in dark or light conditions
600 (6 days). Data represent mean of transcript levels of 3 biological repeats, relative to *PpACT3*
601 and *PpAPT* expression level, ± SE. Asterisks denote significant differences between the dark
602 control and the treatment based on a Kruskal–Wallis test (P < 0.001). (b) Leaf distribution on
603 gametophores from WT (blue dots) *Ppccd8* (orange squares) and *Ppmax2-2* (black triangles).
604 (c) Gametophore height of WT, *Ppccd8*, *Ppmax2-1* phenotype after 25 days under red light
605 (left, scale = 5 mm) and quantifications (right) mean of 3 Magenta, n=43-50 counted
606 gametophores per Magenta. Different letters indicate significantly different results between
607 genotypes based on a *post hoc* Kruskal–Wallis test. (d-e) Transcript levels of red light
608 response markers (*PpHY5* (d) and *PpPOR1* (e)), in WT and *Ppmax2-1* mutants following
609 different times of red light exposure as indicated below the histograms. WL= White Light
610 control. Data represent mean of transcript levels of 3 biological repeats, relative to *PpACT3*
611 expression level, ± SE. Asterisks denote significant differences between the genotypes based
612 on a Kruskal–Wallis test (P < 0.001).

613

614 Fig. 6 The *Physcomitrella patens Ppmax2* mutation is epistatic to *Ppccd8*. (a) Bright field
615 photographs of WT, single *Ppccd8*, single *Ppmax2* mutant and *Ppccd8-Ppmax2* double
616 mutant. Scale: left, 20 day-old: 1mm; right, 2 month-old: 5mm. (b) Comparison of *Ppccd8-*
617 *Ppmax2* mutant plant diameter to that of WT and *Ppccd8* and *Ppmax2* mutants after 4 weeks
618 (mean of 3 plates with 16 plants measured per plate, ± SE). Different letters indicate
619 significantly different results between genotypes based on an ANOVA (P < 0.05) (c)
620 Expression of the SL responsive gene *PpCCD7* relative to *PpAPT* and *PpACT3* expression in
621 *Ppccd8, Ppmax2-1* and *Ppccd8-Ppmax2* grown for 3 weeks in the light. Asterisks denote
622 significant differences between *Ppccd8* and the other mutants based on a *post-hoc* Kruskal–
623 Wallis test (P < 0.001). Data represent means of 3 biological repeats ± SE.

624

625 Fig.7: Expression of *Physcomitrella patens PpMAX2* gene in the *Arabidopsis max2* mutant
626 does not restore MAX2 function (a) Mean height and (c) mean number of rosette branches , ±
627 SE, from 4-week-old *Arabidopsis* plants (n=12) of each genotype 10 days after decapitation.
628 (b) Corresponding pictures of one exemplary plant per genotype are shown. (d) Hypocotyl
629 length of 5-day-old *Arabidopsis* plantlets (n=15) grown in vitro under low light intensity (20-
630 30 µE). Names of the transformed plants indicate the construct harbored. Controls used for all
631 experiments were *Arabidopsis* WT Columbia (Col-0, white bar), *Atmax2-3* mutant (N592836,

632 black bar) and *Atmax2-3* transformed with constructs expressing *AtMAX2* under the control of
633 the pUbi10 promoter. Different letters indicate significantly different results based on a post-
634 hoc Kruskal–Wallis test ($P < 0.05$). Data represent means ± SE.

635

636 Fig. 8: Model for MORE AXILLARY GROWTH2 (MAX2) roles in land plants. In vascular
637 plants, the MAX2 F-box protein is central for shoot branching, seed germination and
638 photomorphogenesis, by mediating Strigolactone (SL), the still unknown KAI2 Ligand (KL)
639 and light signals. D14 and KAI2 are known receptors for SL and KL respectively. In moss,
640 the F-box protein, PpMAX2, is likely involved in photomorphogenesis and plant spread
641 (protonemal growth), but another F-box protein may be required for SL signaling. Receptors
642 for these signals are still to be identified among the numerous moss PpKAI2Like predicted
643 proteins. The similar photomorphogenic phenotypes of *Atkai2* and *Atmax2* mutants suggest
644 that the effect of light on development through MAX2 could, at least in part, be mediated via
645 changes in KL that are perceived by KAI2 (dotted line). Arrows on the left mean signaling
646 mediation. Arrows on the right mean positive action while blunt-ended lines mean repression.

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The following Supporting Information is available for this article:

Fig. S1 Gene targeting of the *PpMAX2* gene

Fig. S2 Expression of PpMAX2 constructs in Arabidopsis

Fig. S3 PpMAX2: Phylogenetic tree, absence of intron, sequence alignment and homology model produced by I-TASSER

Fig. S4 Expression of the *PpMAX2* gene: eFPbrowser data

909 **Fig. S5** Gametophore branching in *Ppmax2*

910 **Fig. S6** Transcriptional response of the *Ppmax2* mutant to (±)-GR24

911 **Fig. S7** High doses of (±)-GR24 application mimics the *Ppmax2* mutant phenotype

912 **Table S1** 5'-3' sequences of primers used in the study

913

Fig. 1 Pattern of *PpMAX2* gene expression and subcellular localization of the protein

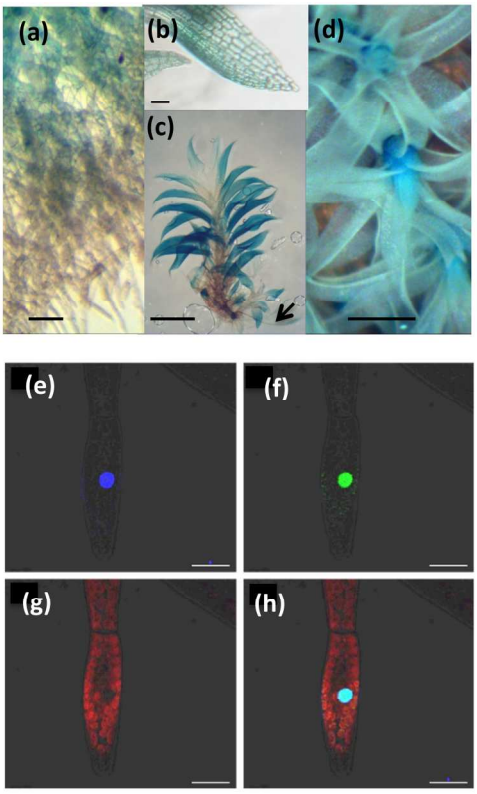

Fig.1

179x178mm (300 x 300 DPI)

Fig. 2 *Ppmax2* mutants are affected in development and show contrasting phenotype to the *Ppccd8* SL synthesis mutant.

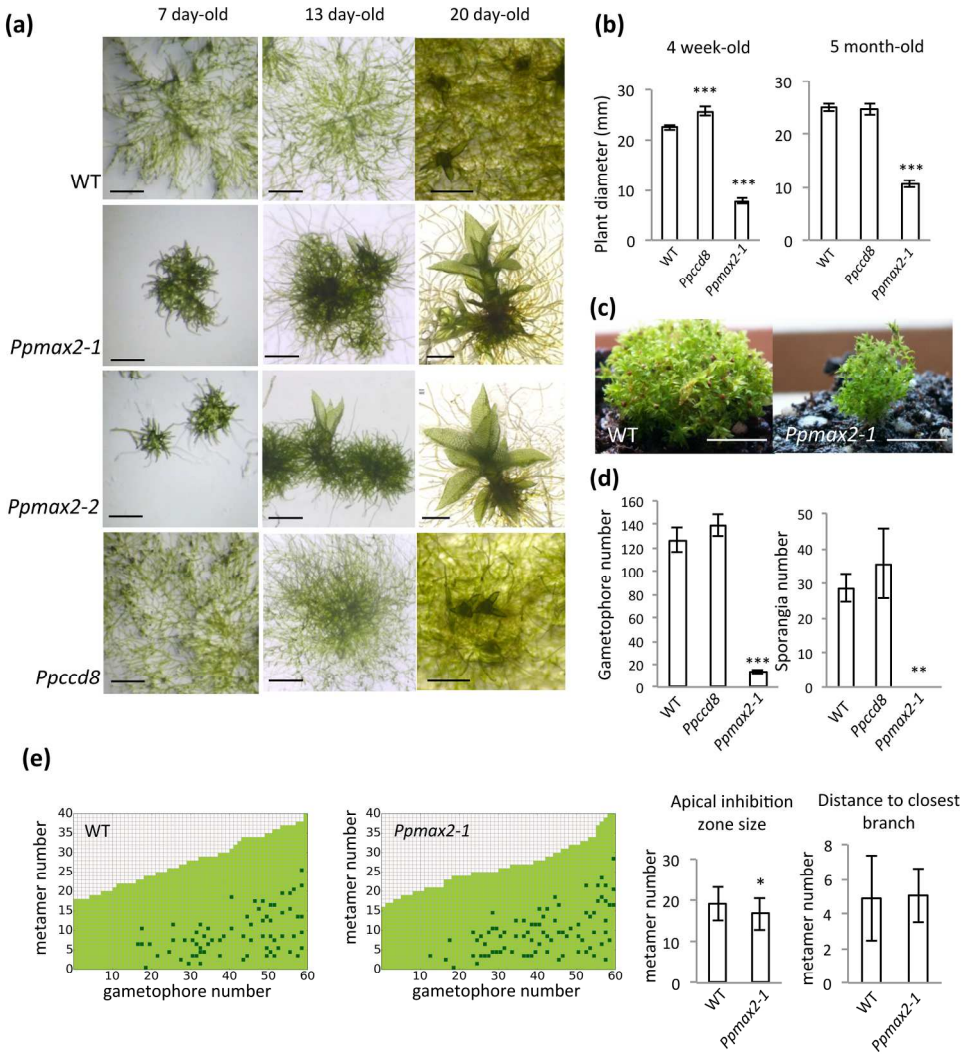

Fig.2

188x224mm (300 x 300 DPI)

Fig. 3 The *Ppmax2* mutant exudate tested on *PpCCD7* expression is similar to WT

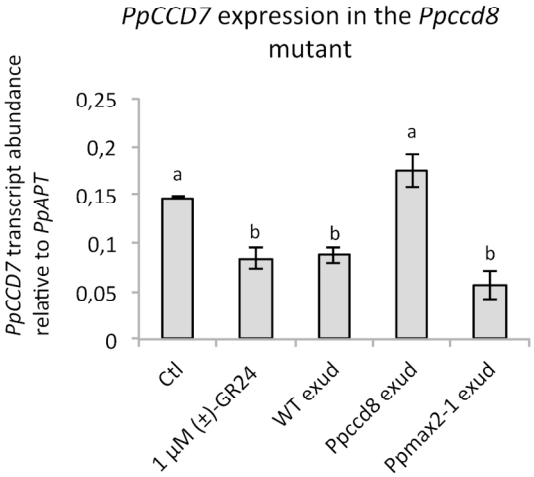

Fig.3

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Fig. 4: the *Ppmax2* mutant is sensitive to the synthetic SL GR24

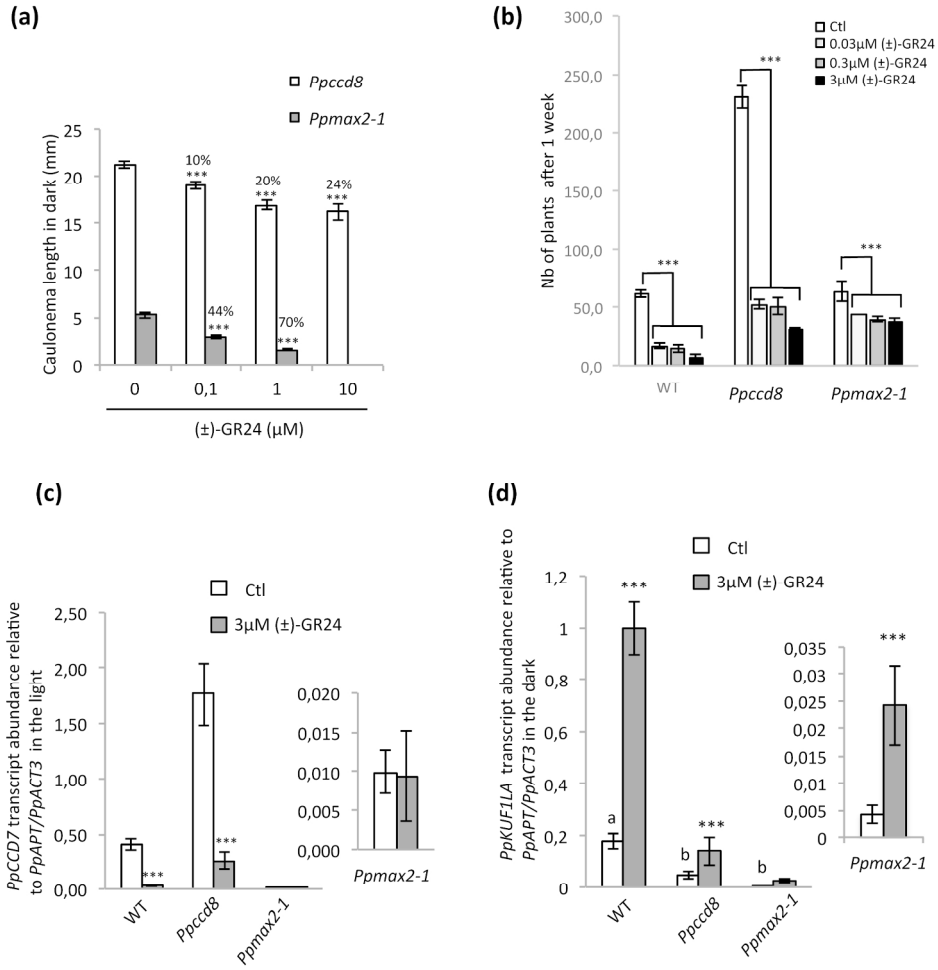

Fig.4

188x206mm (300 x 300 DPI)

Fig. 5: The *Ppmax2* mutant has impaired photomorphogenesis

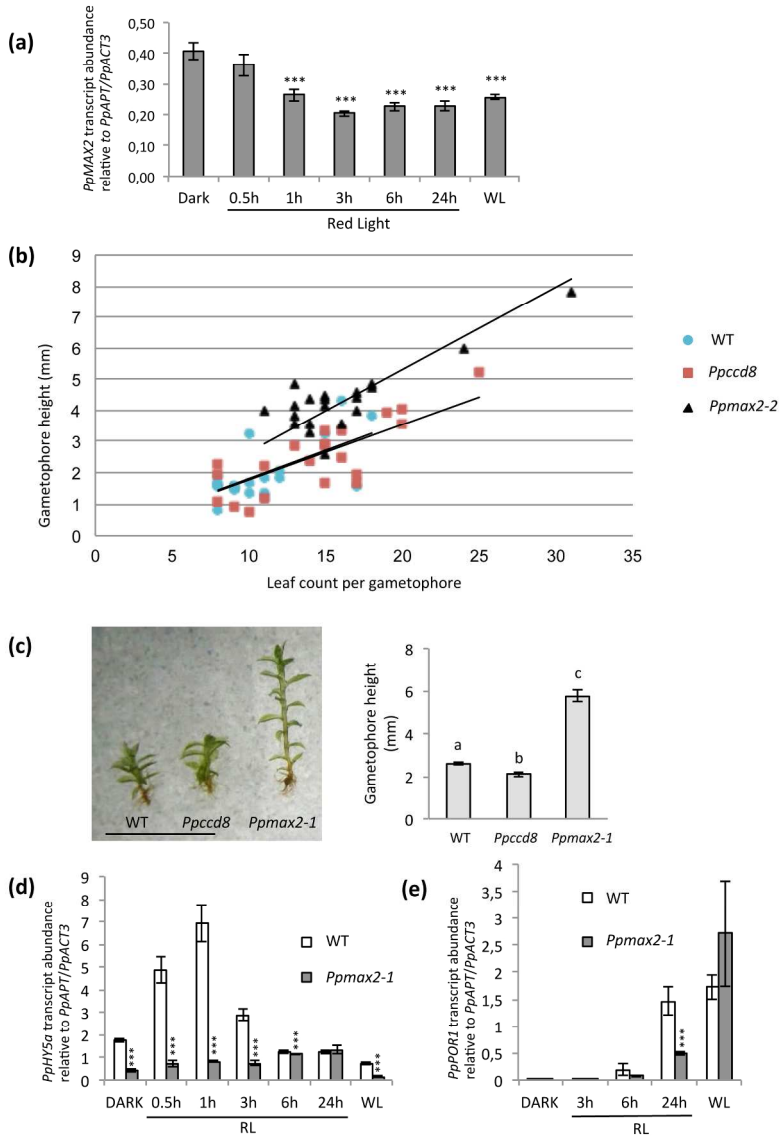

Fig.5

186x252mm (300 x 300 DPI)

Fig. 6 The *Ppmax2* mutation is epistatic to *Ppccd8*

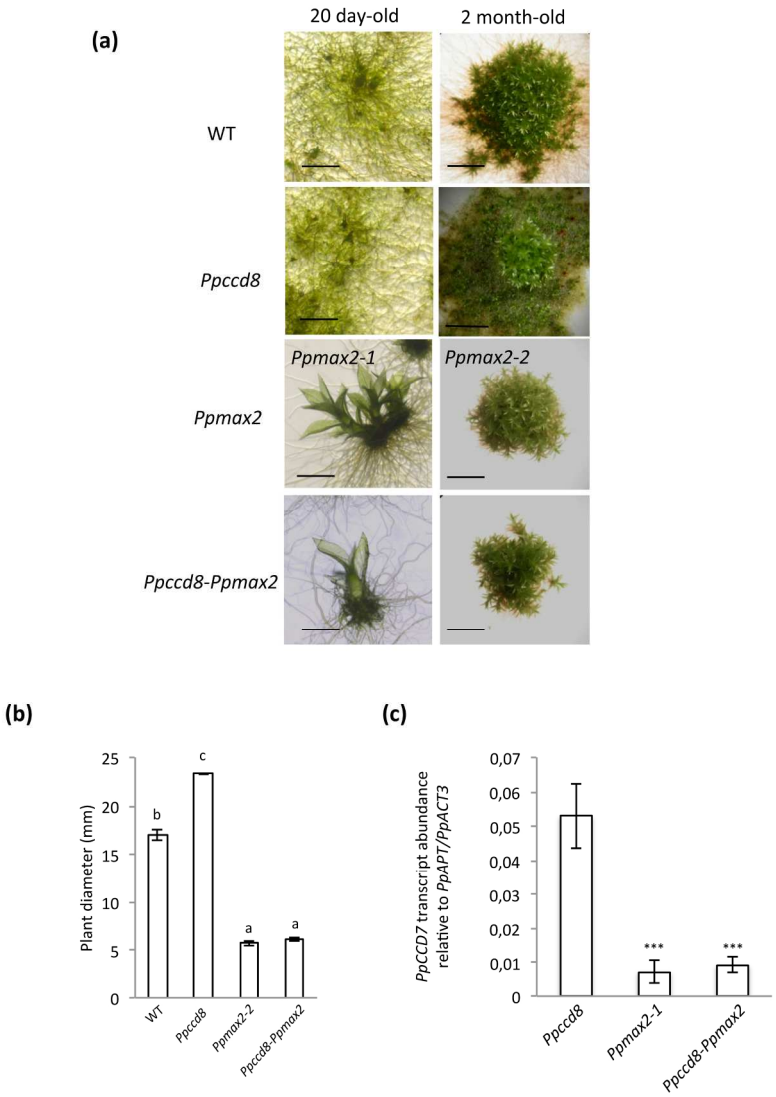

Fig.6

178x243mm (300 x 300 DPI)

Fig. 7: Expression of *PpMAX2* in the *Arabidopsis max2* mutant does not restore MAX2 function.

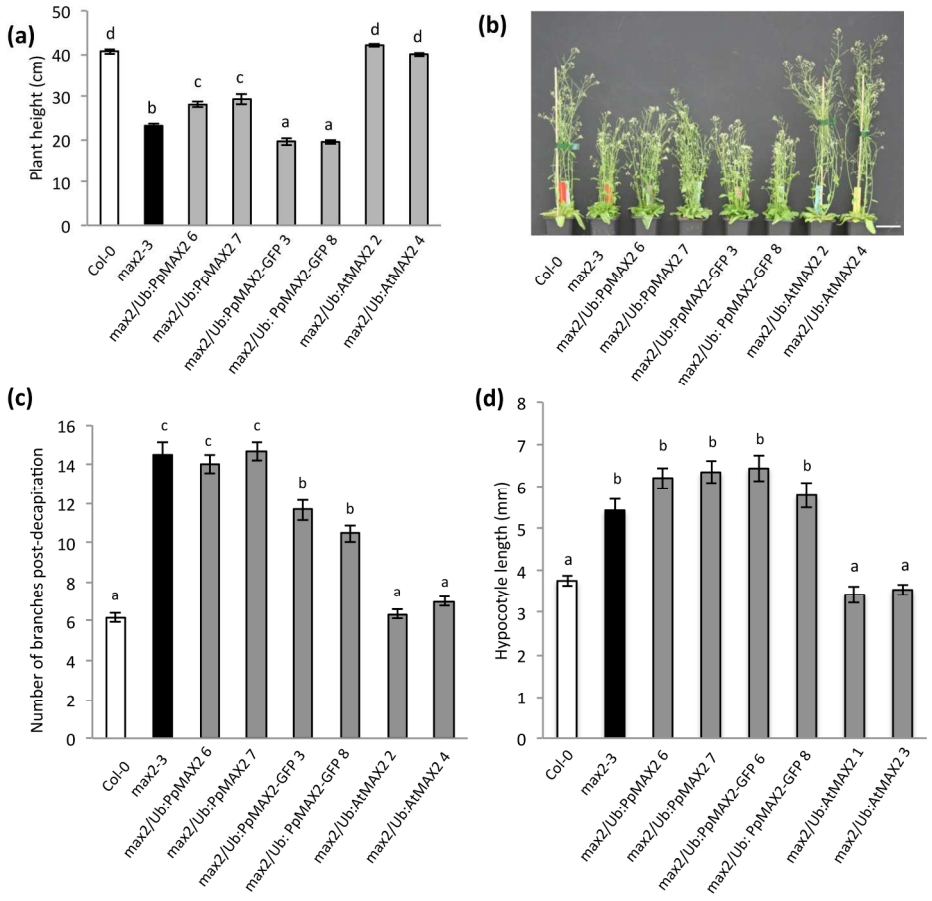

Fig.è

194x205mm (300 x 300 DPI)

Fig. 8: Model for MAX2 roles in land plants

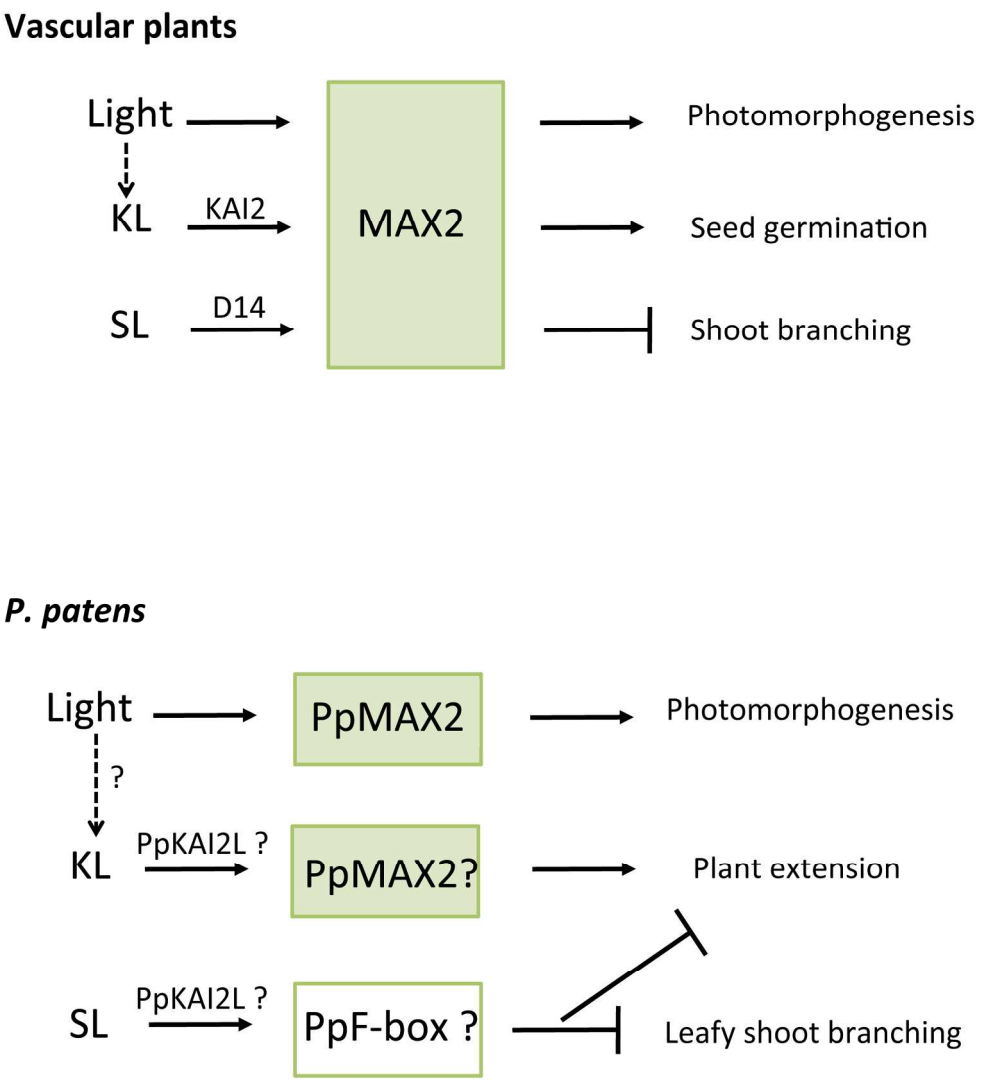

Fig.8

191x225mm (300 x 300 DPI)