Title: Sucrose promotes D53 accumulation and tillering in rice

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Abstract (200 words max):

- Shoot branching, a major component of shoot architecture, is regulated by multiple signals. Previous studies have indicated that sucrose may promote branching through suppressing the inhibitory effect of the hormone strigolactone (SL). However, the molecular mechanisms underlying this effect are unknown.
- Here we used molecular and genetic tools to identify the molecular targets underlying the antagonistic interaction between sucrose and SL.
- We showed that sucrose antagonises the suppressive action of SL on tillering in rice and on the degradation of D53, a major target of SL signalling. Sucrose inhibits the expression of *D3,* the orthologue of the arabidopsis F-box protein MAX2 required for SL signalling. Over-expression of *D3* prevents sucrose from inhibiting D53 degradation and enabled the SL inhibition of tillering under high sucrose. Sucrose also prevents SL-induced degradation of D14, the SL receptor involved in D53 degradation. Interestingly, *D14* over-expression enhances D53 protein levels and sucrose-induced tillering.
- Our results show that sucrose inhibits SL perception by targeting key components of SL signalling and, together with previous studies reporting the inhibition of SL synthesis by nitrate and phosphate, demonstrate the central role played by strigolactones in the regulation of plant architecture by nutrients.

Keywords: strigolactones, sugar hormone interactions, shoot branching, plant architecture

Introduction

Plants are sessile organisms that have evolved the ability to adapt to constantly changing environmental conditions. Shoot branching regulation in angiosperms allows plants to adjust to a given environment, contributes to the overall plant architecture and is considered as an important economic trait for horticulture and agriculture (Wang *et al.*, 2018; Li *et al.*, 2019; Guo *et al.*, 2020). Thus, the study of shoot branching is of major importance for food security, given the increasing global population.

Shoot branching is regulated by different processes including apical dominance, a phenomenon observed in many plants whereby the growing shoot apex inhibits the growth of axillary buds on the same stem. Factors such as herbivory, pruning, and accidental damage to the main shoot break this dominance, allowing lateral buds to grow into branches. Moreover, depending on the environmental conditions, buds can be induced to grow into branches, thus allowing plant architecture to adapt to the prevailing conditions. The shoot tip inhibits axillary bud outgrowth by producing a basipetal flow of auxin in the adjacent stem (Thimann & Bonner, 1933; Domagalska & Leyser, 2011; Barbier *et al.*, 2017) and because the rapid growth of the shoot tip diverts nutrients away from axillary buds (Mason *et al.*, 2014; Rameau *et al.*, 2015; Barbier *et al.*, 2019b).

Part of the inhibitory effect of auxin on shoot branching is mediated by the phytohormones, strigolactones (SL). The apically-derived auxin upregulates the expression of strigolactone synthesis genes in the stem (Domagalska & Leyser, 2011; Rameau *et al.*, 2015). SL are sensed by DWARF14 (D14), an α/β hydrolase (Arite *et al.*, 2009; Hamiaux *et al.*, 2012), which then interacts with the F-box protein DWARF3 (D3), the rice orthologue of the arabidopsis MORE AXILLARY GROWTH2 (MAX2), to form a Skp1-Cullin-F-box (SCF) E3 ubiquitin ligase ($SCF^{D3/MAX2}$) complex (Zhao *et al.*, 2014). DWARF53 (D53) protein is degraded by the SL-mediated ubiquitination and proteasomal degradation through the D14–SCF^{D3} complex (Jiang *et al.*, 2013; Zhou *et al.*, 2013). Gain-of-function *d53* mutants in rice and arabidopsis display highly branched phenotypes, and loss-of-function can recover the highly branched phenotype of mutants deficient in SL levels or signalling (Jiang *et al.*, 2013; Zhou *et al.*, 2013). The TEOSINTE BRANCHED1, CYCLOIDEA, and PCF (TCP) family transcription factor TEOSINTE BRANCHED1 (TB1) acts as a negative regulator of tillering and is known as an important hub, integrating

69 different signals, including SL, that induce *TB1* gene expression (Takeda *et al.*, 2003; Aguilar-
70 Martínez *et al.*, 2007; Minakuchi *et al.*, 2010; Dun *et al.*, 2012; Wang *et al.*, 2019a).

71 Auxin treatment is not always enough to restore apical dominance after shoot tip removal
72 (Cline, 1996; Beveridge, 2000; Morris *et al.*, 2005; Barbier *et al.*, 2021). As demonstrated upon
73 decapitation in pea, auxin depletion in the stem does not correlate with initial bud growth
74 (Morris *et al.*, 2005; Ferguson & Beveridge, 2009). In this species, this initial bud outgrowth
75 has been correlated with rapid remobilisation of carbohydrates towards the buds (Mason *et*
76 *al.*, 2014; Fichtner *et al.*, 2017). Beyond their trophic role, sugars also act as signalling
77 molecules, allowing plants to adjust their metabolism, growth and development to their
78 environment (Rolland *et al.*, 2006; Sakr *et al.*, 2018; Fichtner *et al.*, 2021b). A signalling role
79 for sugars in bud outgrowth and shoot branching has been reported for different species
80 (Takahashi *et al.*, 2014; Barbier *et al.*, 2015b,a). The low abundant metabolite trehalose 6-
81 phosphate (Tre6P), a sucrose-specific signalling molecule (Fichtner & Lunn, 2021),
82 accumulates rapidly in pea buds upon decapitation (Fichtner *et al.*, 2017) and promotes shoot
83 branching in arabidopsis (Fichtner *et al.*, 2021a). The HEXOKINASE1 (HXK1) signalling pathway
84 (Moore *et al.*, 2003) was also recently shown to promote shoot branching in arabidopsis and
85 to interact with both, the CK and SL pathways (Barbier *et al.*, 2021). A recent study comparing
86 transcriptomes of annual and perennial plants correlated the expression of genes involved in
87 carbon starvation with bud dormancy (Tarancón *et al.*, 2017). In different eudicots, sugar
88 supply to the plant can promote shoot branching (Mason *et al.*, 2014; Barbier *et al.*, 2015b;
89 Dierck *et al.*, 2016); however, this has not yet been reported for monocot plants.

90 The interactions among sugars and hormones during the control of bud outgrowth are not
91 yet fully resolved. Recent studies have indicated that sucrose can antagonise the effect of
92 auxin by inhibiting SL perception to promote bud outgrowth (Bertheloot *et al.*, 2020) and that
93 the promotion of growth by cytokinins may only be effective under conditions where sugars
94 are not readily available (Barbier *et al.*, 2015b; Salam *et al.*, 2021). Interactions between
95 sugars and strigolactones have been recently highlighted in rice during the control of shoot
96 architecture by the circadian clock (Wang *et al.*, 2020). Interestingly, sucrose application to
97 single-node cuttings of rose buds can suppress *MAX2* and *BRANCHED1* (*BRC1,* the arabidopsis
98 orthologue of *TB1*) gene expression (Barbier *et al.*, 2015b; Wang *et al.*, 2019b). In sorghum
99 (*Sorghum bicolor*), defoliation and shade treatments, which decrease sugar availability, inhibit

100 bud outgrowth and up-regulate *MAX2* expression (Kebrom *et al.*, 2010; Kebrom & Mullet,
101 2015).

102 The aim of this study was to test whether sugar availability affects SL-induced tillering
103 inhibition in rice and to make the first steps towards identifying the molecular components
104 involved. Using physiological experiments and genetic tools, we sought to identify which
105 components of SL signalling are targeted by sucrose during tillering in rice, and bud outgrowth
106 in pea. Since both sugar and SL levels in plants are controlled by environmental factors, this
107 study will shed light on how environmental factors may regulate branching and tillering at the
108 molecular level.

109

110 Materials and Methods

111 Plant material and growth conditions

112 For rice, available lines created in different backgrounds were used as indicated in the figure
113 legends, and the corresponding wild types were used as controls. The tiller development
114 assay in response to sucrose and GR24 was performed using the *japonica* cultivar
115 HuaiDao5(Patil *et al.*, 2019). The seeds were sterilised as per the method described earlier
116 (Zhao *et al.*, 2014) with slight modifications. In brief, de-husked seeds were sterilised with
117 30% NaClO solution in a shaker for 30 minutes and then washed with sterilised de-ionised
118 water at least five times. The seeds were directly sown on the solidified (0.5% agar) half-
119 strength MS media with different sucrose concentrations with adjusted pH of 5.8. For the SL
120 treatments, rac-GR24 was used in all the experiments (CX23880, Chiralix). The plants were
121 grown on the different sucrose concentrations for three weeks with or without 1 µM GR24
122 under a 16-hr light (200 µmol m^{-2} s^{-1})/8 hr dark cycle at 28°C in a growth chamber. The GR24
123 and the corresponding treatment combinations were replaced at weekly intervals,
124 maintaining strictly sterile conditions.

125 The calli of wild-type (WT) plants (HuaiDao5) grown on NB media plates at 28°C in the dark
126 were used for the D53 protein degradation assay. The *D3* and *D14* over-expressing lines and
127 their corresponding mutant and WT lines were used from the earlier work (Zhao *et al.*, 2014).
128 The lines were maintained in field condition at the experimental station of Shandong Rice

129 Research Institute, Shandong, China. The lines used for sucrose sensitivity assay consisted of
130 *d3* (*s2-215*^{Q393Stop}) (Patil *et al.*, 2019) and *d14* (*htd-2*) (Liu *et al.*, 2009) mutants in the
131 Nipponbare WT background.

132 For pea, decapitation and sucrose petiole feeding experiments were performed on the
133 Torsdag L107 background. *In vitro* sucrose treatment with single nodes was performed as
134 described earlier (Bertheloot *et al.*, 2020) in the *rms3*, *rms4* mutants and their corresponding
135 WT Terese. For the decapitation experiment, plants were grown in a glasshouse with a
136 controlled environment (Fichtner *et al.*, 2017). For the sucrose petiole feeding experiment,
137 plants were grown in a growth chamber with 16 hrs of light (125 µmol m^{-2} s^{-1}) at a temperature
138 of 22°C during the day and of 20°C at night.

139

140 **Rice callus induction**

141 Husks were removed from rice seeds which were then sterilized with the 30% NaClO solution
142 and washed for multiple times with sterilized deionized water. The seeds were then placed
143 on solidified NB media petri-plates in the dark at 28°C for one week. The embryonic calli were
144 then separated and multiplied on fresh NB media plates maintained at 28°C in the dark. To
145 prepare 1 L NB medium, 4.1 g NB basal medium (Phytotech lab), 2 g Casein hydrolysate, 4 g
146 L-Proline, 2 g L-Glutamine, 200 µl 2-4-Dichlorophenoxyacetic acid (10 mg ml^{-1}), 30 g sucrose
147 (adjusted depending on experimental needs) and 3 g Phytagel were mixed together (pH =
148 5.8).

149

150 **Protein quantification assay using callus tissues and tiller buds**

151 The calli were grown on NB media plates containing different sucrose concentrations. Around
152 250 mg callus tissue was ground in liquid nitrogen, using a mortar and pestle to make a fine
153 powder. The powder was transferred to 1.5 ml microcentrifuge tubes and mixed with 250 µl
154 of ice-cold TBT buffer (100 mM AcOK, 20 mM KHEPES pH 7.4, 2 mM MgCl2, 0.1% Tween-20,
155 1 mM DTT, and 0.1% protease inhibitor cocktail). The antibody preparation, samples
156 preparation, and protein blots were developed as per the method described earlier (Jiang *et*

157 *al.*, 2013). The D53-specific polyclonal antibody produced in mouse was used for immune
158 detection following 1:1000 dilution in non-fat dairy milk. Anti-HSP82 or anti-actin was used
159 as a loading control following 1:3000 dilution. Horse-radish peroxidase-conjugated anti-
160 mouse lgG was used as a secondary antibody (CWBIO, Beijing, China) following 1:3000
161 dilution. The western blots were developed using Tanon™ High-sig ECL western blotting
162 Substrate (Cat. no:180-5001) with a Tanon 6100 chemiluminescent imaging system. For each
163 Western blot, the band intensity of the target and loading control proteins was determined
164 using the "Gels" analysing tool in ImageJ. The values of the target proteins were then
165 normalized by the values of the loading control, and the values were presented relative to the
166 control condition for each genotype to ease comparison across treatments.

167

168 **Gene expression analysis**

169 For rice, shoot base tissues (0.5 cm) were harvested from two-week-old seedlings grown
170 hydroponically. Total RNA was isolated using RNAprep Pure Plant Kit (Tiangen, Beijing, China,
171 cat. no. DP432) following the manufacturer's instructions. Reverse transcription of 500 ng
172 RNA was performed using the Vazyme, HiScript II Q Select RT SuperMix for qPCR (cat no.
173 R233). Real-time quantitative PCR was performed by Vazymes Cham Q QPCR reagent kit (cat
174 no. Q331) using the ABI Prism 7500 Sequence Detection System as per the program
175 recommended by both the instrument and the reagent company. Transcript levels were
176 detected by CT values relative to *ACTIN1* as a reference gene. All the primer sequences used
177 in this study are listed in Supplementary Table S1. Gene expression in pea buds was monitored
178 using a phenol/chloroform free CTAB-based method as described earlier (Barbier *et al.*,
179 2019a).

180

181 ***Nicotiana benthamiana* leaf agroinfiltration and *in vitro* luciferase activity assay**

182 The pCambia1200 vector was modified by integrating Firefly luciferase and Renilla luciferase
183 coding sequences driven by the CaMV35S promoter in addition to the hygromycin resistance
184 marker. The vector then called pCambia1200 35S-LUC had both transient and stable
185 expression capabilities (Figure S2) (Sun *et al.*, 2021). The coding sequence of the *D53* gene

186 was amplified using D53 LUC-F-GGGCGGAAAGGAATTCATGCCCACTCCGGTGG and D53 LUC-R-
187 TAGATCCGGTGGATCCTCAACAATCTAGAATTATTCTTGGCGGGAG primer pairs and cloned at the C-
188 terminal region of the Firefly luciferase gene using *Eco*RI and *Bam*HI restriction sites following
189 the In-fusion® cloning system by Clonetech, Takara biotech, Japan. The plasmids were
190 transformed into the Agrobacterium strain EHA105 to transfect into *Nicotiana benthamiana*
191 leaves for the transient expression of the D53-Firefly luciferase fused protein. This vector also
192 consists of a Renilla luciferase gene as an internal control which was used to quantify the
193 relative amount of the D53 protein levels. The Agrobacterium strain was transfected into the
194 *N. benthamiana* leaves as per the methods described earlier (Chen *et al.*, 2008) . To check the
195 effects of sucrose and GR24 on the transiently expressed D53 levels, different treatment
196 combinations were infiltrated directly into the leaves already transfected with the
197 Agrobacterium strain harbouring the luciferase-fused D53 construct. The leaves were then
198 harvested in liquid nitrogen, and the activities of Firefly luciferase and Renilla luciferase were
199 determined using the Dual-luciferase® reporter assay system from Promega (cat. no. E1910).
200 The LUC activity was calculated by normalising Firefly luciferase values with Renilla luciferase
201 and was presented as relative luciferase values.
202

203 ## Results

204 **Sucrose and strigolactones interact antagonistically to regulate tillering in rice**

205 In dicotyledonous models, sucrose has been shown to promote axillary bud outgrowth and
206 alleviate the inhibitory effect of SL during this process (Dierck *et al.*, 2016; Bertheloot *et al.*,
207 2020). However, this antagonistic interaction has not been reported in monocotyledons
208 plants, which have a different architecture compared with eudicots, and are evolutionarily
209 distant. To evaluate sucrose and SL effects on tillering bud outgrowth in rice, we grew wild-
210 type (WT) Huaidao-5 plants hydroponically with different sucrose concentrations with or
211 without 1 µM rac-GR24 (a synthetic SL analogue). To be in a physiological range of sucrose
212 concentrations, we added up to 4% sucrose (~120 mM), which is lower than the endogenous
213 sucrose levels reported in rice (between 200 mM and 600 mM) (Hayashi & Chino, 1990). We
214 observed that sucrose triggered bud elongation in a dose-dependent manner with or without
215 addition of rac-GR24 (Figure 1). In the absence of sucrose, tiller bud elongation remained
216 suppressed. With 2% sucrose, GR24 strongly inhibited tiller bud elongation (73% inhibition)

217 (Figure 1B). However, with 4% sucrose, the inhibitory effect of GR24 on tiller bud elongation
218 was reduced to 30% inhibition. A two-way ANOVA demonstrated that the effect of GR24
219 largely depends on the concentration of sucrose (*p*-value = 0.000019). In contrast to sucrose,
220 sorbitol only had a very small promoting effect on tillering and barely alleviated the inhibitory
221 effect of GR24, showing that the effect of sucrose was largely independent of an osmotic
222 effect (Supplementary Figure S1). These phenotypic data support a similar hypothesis as
223 proposed previously for selected eudicots, namely that sucrose and SL interact
224 antagonistically to regulate tillering.

225

226 **Sucrose inhibits GR24-induced degradation of D53 protein in rice**

227 The D53 protein and its orthologues in arabidopsis SMXL6, 7 and 8 play a crucial role in SL-
228 mediated shoot branching in rice (Jiang *et al.*, 2013; Zhou *et al.*, 2013) and arabidopsis
229 (Soundappan *et al.*, 2015; Wang *et al.*, 2015), respectively. Since sucrose reduces the SL
230 response, we proposed that sucrose might promote D53 accumulation. We therefore tested
231 the impact of sucrose on D53 accumulation. Since dormant tiller buds are very small and D53
232 protein levels are difficult to detect in shoot tissues, we first used rice calli, which have
233 previously been successfully used for this purpose (Jiang *et al.*, 2013). In the absence of GR24
234 in the growth medium, the D53 protein levels strongly increased with the increasing sucrose
235 concentration (Figure 2A)

236 We then tested the effect of sucrose on D53 in the presence of GR24. As the calli grown on
237 different sucrose concentrations accumulate different levels of D53, calli grown on 4%
238 sucrose plates showing a similar amount of D53 protein were used. The calli were washed
239 twice with sterile water to remove the exogenous sucrose before being transferred to liquid
240 media containing either no sucrose, or 4% sucrose. After 30 minutes of stabilisation, 0.5 µM
241 GR24 was supplemented to the 0% and 4% sucrose treatments. In the absence of sucrose,
242 D53 protein was degraded within 5 minutes of treatment with GR24. However, in the
243 presence of 4% sucrose, it took 20 minutes for GR24 to lead to a similar degradation of the
244 D53 protein (Figure 2B).

245 To confirm this result, we tested OsD53 degradation in response to sucrose and GR24 in
246 *Nicotiana benthamiana* leaves transiently expressing an *OsD53* coding sequence fused to a
247 LUCIFERASE (LUC) reporter (Supplementary Figure S2). D53 protein accumulation was
248 assessed by measuring the LUC activity normalised with Renilla luciferase values. Without
249 GR24, sucrose had a minor effect on LUC activity which was only significantly enhanced by
250 sucrose at 8 hrs. In the absence of sucrose, the LUC activity was lower in the presence rather
251 than in the absence of GR24 at 4 hr and 8 hr after hormone or control treatment. However,
252 in the presence of 4% sucrose, the LUC activity was similar with or without GR24 (Figure 2C).
253 Again, these observations show that D53 protein levels are maintained at higher levels in the
254 presence of sucrose and that the degradation rate of D53 in response to GR24 is lower under
255 these sucrose conditions.

256 To observe whether results from calli and agroinfiltrated *N. benthamiana* may be relevant *in*
257 *planta*, we measured D53 proteins levels in dormant tiller buds (length < 3 mm) harvested
258 from plants fed hydroponically with 4% sucrose for one hour, and in which tiller buds were
259 only subjected to the plant's endogenous SL levels. The result indicated that supplying 4%
260 sucrose to rice plants grown hydroponically promoted the accumulation of the D53 protein
261 in dormant tiller buds (Figure 2D), further supporting the hypothesis that sucrose promotes
262 D53 accumulation during tillering regulation.

263

264 **Sucrose inhibits the expression of genes involved in strigolactone signalling**

265 D53 promotes the outgrowth of tillers by inhibiting the expression of the TCP transcription
266 factor gene *TB1* (Takeda *et al.*, 2003; Minakuchi *et al.*, 2010). Given the accumulation of D53
267 proteins in response to sucrose (Figure 2), we predicted that sucrose treatment should
268 suppress *TB1* expression. To test this, we measured *TB1* expression in rice calli treated with a
269 range of sucrose concentrations. The results show that sucrose inhibited the expression of
270 *TB1* in rice callus tissues in a dose-dependent manner (Figure 3A).

271 We then determined whether the change in D53 protein levels could be explained by a change
272 in *D53* expression at the transcript level. Our results indicate that, in contrast to *TB1*
273 expression and the D53 protein level, *D53* gene expression is not responsive to sucrose (Figure

274 3B). These observations suggest that sucrose promotes D53 protein accumulation through a
275 post-transcriptional mechanism.

276 We then tested whether the observations made on calli were relevant to tissues where the
277 regulation of tillering occurs. To do so, we harvested dormant tiller buds (length < 3 mm) and
278 shoot bases (length = 5 mm), enriched in buds and stem. As in calli, sucrose treatment through
279 hydroponic media down-regulated the expression of *TB1* (Figure 3A) in tiller buds and in shoot
280 bases. The expression of *D53* was not affected by sucrose in calli or in the shoot base, but was
281 repressed by 4% sucrose in tiller buds. This is consistent with the negative correlation
282 reported for D53 protein level and *D53* gene expression (Zhou *et al.*, 2013).

283 Given that sucrose reduces SL response in buds (Dierck *et al.*, 2016; Bertheloot *et al.*, 2020)
284 (Figure 1) and enhances the level of D53 protein (Figure 2), we predicted that sucrose may
285 affect components of the SCF complex formed by D14 and D3, which are required for D53
286 protein degradation (Zhou *et al.*, 2013; Zhao *et al.*, 2014). Consistent with such a role of D3
287 and D14 in sucrose regulation of D53, *D3* and *D14* gene expression was significantly reduced
288 by sucrose in calli, tiller buds and shoot bases, although the inhibition of *D14* in shoot bases
289 was milder compared to the inhibition of *D3* expression in this tissue (Figure 3C,D).

290 We then tested whether sucrose could also directly regulate D14 or D3 protein levels. To do
291 so, we tested the impact of 4% sucrose on D14 and D3 accumulation in calli of transgenic lines
292 over-expressing HA-tagged *D14* or *D3* driven by the constitutively active *OsACTIN1* promoter
293 (*ACTp*) in the *d14* and *d3* mutant background, respectively (Supplementary Figure S3). In
294 these lines, we expected stable synthesis of the tagged proteins. To establish this approach,
295 we first observed that the HA-tagged over-expressing lines complemented the tiller number
296 and almost fully complemented the plant height phenotypes of the corresponding *d14* and
297 *d3* mutants (Supplementary Figure S4). In the absence of sucrose, GR24 led to the almost
298 complete degradation of the D14 protein over 12 hrs in the HA-D14 over-expression line
299 (Figure 3E). This indicates that the GR24-induced degradation rate of the HA-D14 protein in
300 the absence of sucrose exceeds the stable rate of HA-D14 synthesis in this line. Strikingly, in
301 the presence of sucrose, GR24 did not affect the levels of HA-D14 (Figure 3E). This indicates
302 that sucrose antagonises GR24-induced degradation of D14. In contrast, D3 protein levels in
303 the HA-D3 over-expression line were quite stable in response to GR24 and sucrose (Figure

304 3F). Altogether, this suggests that sucrose inhibits the GR24-induced D14 degradation and
305 does not directly regulate D3 protein levels.

306

307 **D3/OsMAX2 over-expression prevents the regulation of D53 by sucrose**

308 If sucrose acts via D14 and/or D3 to regulate D53 protein accumulation as indicated above
309 (Figure 3A-D), we would predict that over-expression of one or both of these two genes would
310 prevent sucrose from promoting D53 accumulation and may prevent sucrose-induced
311 tillering. To test the sucrose response over a short time frame, we grew the calli of *ACTp*:*D3,*
312 *ACTp*:*D14* over-expression lines on NB medium plates supplemented with 1% sucrose. Since
313 these lines have been created in different backgrounds (GSOR300002 and GSOR300192,
314 respectively), these cultivars were used as controls. The calli were then washed and rinsed for
315 60 min with sterile water. The calli were then shifted to liquid media containing 4% sucrose
316 and collected after 0, 2, 4 and 8 hrs to determine D53 protein levels. In two WT backgrounds,
317 an increase in D53 levels was observed in response to sucrose (Figure 4A,B). Constitutive over-
318 expression of *D14* or *D3* diminishes the response of D53 to sucrose but in two different and
319 opposite ways. *D14* overexpression causes a high level of D53 protein, regardless of the
320 sucrose supply. In contrast *D3* overexpression prevents accumulation of D53 under sucrose
321 treatment. These results indicate that *D3* over-expression prevents sucrose-induced D53
322 accumulation, while *D14* over-expression mimics the effect of sucrose supply on D53 protein
323 levels.

324 Since sucrose and SL have an antagonistic effect on D53 protein levels (Figure 2B-C), we
325 measured D53 protein levels in rice calli over-expressing *D14* and *D3* in response to both GR24
326 and sucrose. As observed in Figure 2, the effect of GR24 on D53 degradation was delayed by
327 sucrose treatment in the WTs (Figure 4C and 4E). *D14* overexpression did not prevent the
328 promoting effect of sucrose on D53 levels (Figure 4D). In contrast, *D3* over-expression led to
329 total degradation of D53 after 5 minutes of GR24 treatment, regardless of the sucrose
330 concentration in the medium (Figure 4F). These results suggest that *D3* over-expression, but
331 not that of *D14*, prevents sucrose from antagonising the SL-induced D53 degradation.

332 Similar findings to those obtained in rice calli were also observed in *N. benthamiana* leaves.
333 In this system the native *N. benthamiana MAX2/D3* expression was also inhibited by sucrose
334 (Figure 4G). We thus tested whether over-expressing *D3* in *N. benthamiana* leaves would also
335 prevent sucrose from antagonising the effect of SL on D53 accumulation. We therefore
336 followed the same procedure as described in Figure 2C and co-transfected D53-LUC with a
337 *35Sp:D3* construct into *N. benthamiana* leaves. In this system, 4% sucrose almost totally
338 alleviated the effect of GR24 on D53 degradation when D53-LUC was solely transfected
339 (Figure 4H). However, when the LUC-D53 construct was co-transfected with the *35Sp:D3*
340 over-expressing construct, sucrose could not prevent the negative effect of GR24 on D53
341 levels (Figure 4H). The same trend was also observed in a second independent experiment
342 (Supplementary Figure S5). These results further support the hypothesis that sucrose
343 alleviates the inhibitory effect of GR24 on D53 protein levels by inhibiting *D3/MAX2*
344 expression.

345 We then tested whether sucrose could still have a promoting effect on D53 levels in the *d3*
346 mutant. To do so, we grew WT and *d3* rice calli on 4% sucrose for three weeks, rinsed them
347 with water and transferred them on a range of sucrose concentrations with 0.5 µM GR24 for
348 10 min. The result shows that D53 accumulates a lot more in the *d3* than in the WT
349 background, as expected in presence of GR24 (Figure 4I). However, we could observe a strong
350 accumulation of D53 in response to sucrose in the WT calli, while no obvious pattern was
351 observed in the *d3* background. This suggests that the positive effect of sucrose on D53
352 accumulation is visible only if D3 is functional, at least in presence of SL and on this short time-
353 frame.

354 To further investigate whether sucrose could act through D3-independent pathways to
355 regulate D53 protein levels, we measured D53 in WT and *d3* calli grown on a range of sucrose
356 for six weeks without SL (Figure 4J). In these conditions we could observe a positive effect of
357 sucrose in both backgrounds. We also measured *TB1* expression in WT, *d3* and *d14* calli fed
358 for 24 hrs with a range of sucrose concentrations without SL (Supplementary Figure S6). The
359 results indicate that *TB1* expression remains responsive to sucrose in the three backgrounds.
360 Altogether, these results suggest that sucrose may also act through D3-independent
361 pathway(s) to regulate D53 protein levels and *TB1* expression in calli, at least without
362 exogenous SL supplementation.

363 ***D3/OsMAX2* over-expression prevents the promoting effect of sucrose on tillering**

364 Given that D53 promotes tillering (Jiang *et al.*, 2013; Zhou *et al.*, 2013) and that over-
365 expression of *D3*, but not *D14*, prevents the sucrose-induced accumulation of D53 (Figure 4),
366 we tested whether *D3* overexpression in particular would prevent sucrose-induced tillering.
367 WTs and lines over-expressing *D3* and *D14* were grown on 0%, 0.5%, 2% and 4% sucrose
368 media with or without 1 µM GR24 for three weeks. As recorded in our previous experiment
369 (Figure 1), 4% sucrose could antagonise the inhibitory effect of GR24 on tiller bud elongation
370 in the WT lines (Figure 5 A,C). As observed in Figure 1B, two-way ANOVA comparison
371 demonstrated that the impact of GR24 significantly depends on sucrose in the two WT
372 backgrounds (*p*-value = 0.00004 for GSOR300192 and *p*-value = 0.0348 for GSOR300002). This
373 antagonistic effect between GR24 and sucrose was also observed in the *D14* over-expression
374 line (Figure 5B). Strikingly, *D14* over-expression strongly increased the effect of sucrose on
375 tiller length (Figure 5 A,B). In contrast with WT and the *D14* over-expression line, *D3* over-
376 expression inhibited the bud response to sucrose most prominently in presence of GR24 (77%
377 inhibition with 4% sucrose) (Figure 5D). Multiple-way ANOVA comparison revealed that the
378 interaction between sucrose and GR24 did not depend on D14-overexpression (*p*-value =
379 0.33), whereas it showed that this interaction is highly dependent on D3 over-expression *p*-
380 value = 0.000002). Our data indicate that *D3* over-expression inhibits tiller bud elongation in
381 the absence of sucrose, contrasting with results observed in arabidopsis (Stirnberg *et al.*,
382 2007). The difference may be due to the fact that, in the study in arabidopsis, the branching
383 phenotype was recorded as the number of branches at the end of the plant's life, whereas
384 our study in rice captured branching at the beginning of the tiller development. Altogether,
385 these results indicate that *D3* over-expression prevents sucrose from alleviating the inhibitory
386 effect of SL on tillering and that *D14* over-expression promotes sucrose-induced tiller bud
387 elongation.

388

389 **Strigolactone signalling mutants are less sensitive to low sucrose concentrations and remain**
390 **responsive to sucrose.**

391 Our results suggest that the inhibition of tillering in response to low sucrose is due to high
392 strigolactone signalling. If it holds true, the strigolactone signalling mutant *d14* and *d3* should

393 be less inhibited by low sucrose concentrations. We therefore tested this by measuring the
394 response of the loss-of-function *d3* and *d14* mutants (Supplementary Figure S7) to different
395 sucrose concentrations supplied hydroponically (Figure 6). The buds of the *d3* mutant
396 responded more than the WT to low sucrose concentrations including 0% exogenous sucrose
397 (Figure 6A,C,D). The buds of *d14* mutant also grew better on 0% sucrose than the WT.
398 However, the length of *d14* tiller buds were much smaller than the buds of *d3,* and also
399 showed enhanced growth on the difference sucrose concentrations, but this effect was not
400 always significantly different from the WT and was always lower than *d3*. We could also
401 observe that the buds of *d14* and *d3* mutants were still responsive to the increase in sucrose
402 concentration. This observation is in line with our results showing that sucrose could promote
403 D53 accumulation in a D3-independent manner in absence of exogeneous SL (Figure 4J).
404 Altogether, these data indicate that disrupting SL signalling components, especially D3,
405 decreases the sensitivity to low sucrose concentrations and that sucrose also promotes
406 tillering independently of D14 and D3.

407

408 ***RMS4/PsD3/PsMAX2* is involved in the sucrose-induced bud outgrowth in garden pea**

409 The role of sugars in bud release has been well described in garden pea, which is an
410 established model eudicot for the study of shoot branching. In this species, decapitation
411 triggers bud outgrowth through redistribution of sugars towards axillary buds, and sucrose
412 feeding can trigger bud release (Mason *et al.*, 2014; Fichtner *et al.*, 2017). We explored
413 whether a similar mechanism to what we observed in rice may occur in pea. To do so, we
414 examined the expression of *PsD3 and PsD14* in pea (also known as *RMS4/PsMAX2* and *RMS3*,
415 respectively) as well as their downstream target *PsBRC1*, the pea orthologue of *TB1*, in
416 response to sucrose feeding and decapitation (Figure 7). Similar to the strongly sucrose-
417 responsive expression of *D3* and *TB1* in rice, decapitation led to a decrease in *PsD3* and
418 *PsBRC1* expression but not in that of *PsD14* (Figure 7A-C). Additionally, compared with
419 sorbitol used as an osmotic control, sucrose feeding for 4 hrs through the petiole strongly
420 inhibited *PsD3* expression (70%) and, to a lesser extent, *PsD14* expression (50%) (Figure 7D).
421 *PsBRC1* expression was also repressed by sucrose but not significantly, compared with
422 sorbitol at this time point (Figure 7D). The downregulation of expression of the sugar-

423 repressible marker gene *PsDARK INDUCIBLE1* (*PsDIN1*) (Fujiki *et al.*, 2001) indicates that the
424 sucrose fed through the petiole reached the bud at 4 hrs. Altogether, these results support
425 the hypothesis that *RMS4*/*PsD3*/*PsMAX2* is regulated by sucrose during bud outgrowth in
426 pea, similar to that observed for *D3* in rice.

427 We then tested responsiveness to sucrose of the *d14* and *d3* mutants in pea, also known as
428 *rms3* and *rms4*, respectively. To achieve this, we grew pea single nodes on half-strength MS
429 media supplemented with different sucrose concentrations (Figure 7E). Decreasing sucrose
430 concentration from 50 mM to 30 mM inhibited bud elongation of the WT plants. As observed
431 in rice, buds of the *d3* mutant were not as sensitive as the WT to lower sucrose concentrations
432 (30 mM). However, contrary to what we observed in rice, buds of the *d14* mutant were not
433 significantly different from the WT. Altogether these results indicate that disruption of *D3*
434 leads to a lower bud response to decreased sucrose availability.

435

436 ## Discussion

437 **Sucrose antagonises the inhibitory effect of strigolactones on tillering**

438 Sucrose and SL play a crucial role in shaping plant architecture through their antagonistic
439 action on bud outgrowth, as previously demonstrated in dicotyledonous plants like rose, pea
440 and chrysanthemum (Dierck *et al.*, 2016; Bertheloot *et al.*, 2020). In the present study, we
441 demonstrated that sucrose also promotes tillering and inhibits the impact of SL on this
442 process in monocotyledonous plants. In rice, as in the previously mentioned species, the
443 inhibitory effect of SL on bud outgrowth was almost totally prevented by high sucrose
444 concentrations. The inhibitory effect of sucrose on SL perception is not limited to shoot
445 branching as reported by recent studies showing that sucrose can also alleviate the effect of
446 SL on dark-induced leaf senescence in rice (Takahashi *et al.*, 2021) and bamboo (*Bambusa*
447 *oldhamii*) (Tian *et al.*, 2018).

448 The expression of the TCP transcription factor *BRC1* that inhibits shoot branching (Takeda *et*
449 *al.*, 2003; Aguilar-Martínez *et al.*, 2007; Braun *et al.*, 2012), has previously been reported to
450 be repressed by sucrose in dicot species (Mason *et al.*, 2014; Barbier *et al.*, 2015b; González-
451 Grandío *et al.*, 2017; Otori *et al.*, 2017; Wang *et al.*, 2019b). Our observations have

452 demonstrated that the expression of *TB1*, the *BRC1* homologue in monocots, is also repressed
453 by sucrose in rice. Furthermore, D53 protein levels, which inhibit *TB1* gene expression, are
454 increased by sucrose (Figure 2 A,D). Results in rice and *N. benthamiana* showed that SL-
455 mediated D53 degradation was reduced by sucrose treatment (Figure 2 B,C; Supplementary
456 Figure S5). This supports the hypothesis that the antagonistic effect of sucrose on SL-
457 mediated bud inhibition (Dierck *et al.*, 2016; Bertheloot *et al.*, 2020) (Figure 1) is at least partly
458 mediated through sucrose dampening SL-induced D53 degradation (Figure 8).

459

460 **D3/MAX2 plays a key role in the antagonism between sugar availability and strigolactones**

461 The F-box protein D3/RMS4/MAX2 plays an essential role in mediating the SL-dependent
462 degradation of D53 protein through the SKP1–CULLIN–F-BOX (SCF) ubiquitin-proteasome
463 system (Zhou *et al.*, 2013; Zhao *et al.*, 2014). Our data showed that over-expression of *D3*, but
464 not of *D14*, prevented sucrose to antagonise the SL-induced D53 degradation and tillering
465 inhibition (Figures 4 and 5), demonstrating the importance of D3 in modulating the tillering
466 response to SL and sugar availability. In addition, the relative impact of sucrose on D53
467 accumulation in presence of SL was much stronger in the WT than in the *d3* mutant (Figure
468 4I), supporting this conclusion. As previously observed in sorghum, rose and arabidopsis
469 (Kebrom *et al.*, 2010; Barbier *et al.*, 2015b, 2021), sugar availability suppresses *D3/MAX2* gene
470 expression in rice and pea and this effect is stronger and more consistent than for *D14* (Figure
471 3C, 3D and 7D). In addition, we did not observe a direct effect of sucrose on D3 protein levels
472 (Figure 3F), showing that sucrose preferentially regulates *D3* transcription. However, sucrose
473 might also act through D3 protein by regulating the switch between the two conformational
474 states of this protein which has been reported to modulate the binding affinity between D3
475 and D14 (Shabek *et al.*, 2018).

476 Some evidence suggest that D3/MAX2 may retain a function independent of SL. In field
477 conditions, the *d3 (s2-215)* mutant (Patil *et al.*, 2019) showed a more severe dwarf and high
478 tillering phenotype compared with the *d14 (htd-2)* mutant (Liu *et al.*, 2009), both being loss-
479 of-function mutants developed from the same background (Nipponbare) (Supplementary
480 figure S7). In both rice and pea buds, we could also observe this difference between *d14* and
481 *d3* mutants, particularly under low sugar availability (Figure 6 and 7). This stronger impact of

482 *D3* mutation compared with *D14* mutation or SL-deficiency has been reported in different
483 species and in different developmental processes (Umehara *et al.*, 2008; Hayward *et al.*,
484 2009), showing that our observations are not due to a specific allele, and the difference
485 between *D3* and *D14* mutations is conserved in diverse species. It was previously reported in
486 arabidopsis, that over-expression of *MAX2* could partially suppress decapitation-induced
487 branching in a SL-deficient background (Stirnberg *et al.*, 2007), supporting the hypothesis that
488 D3/MAX2 may retain a function independently of SL.

489 Besides mediating SL signalling, D3/MAX2 has been shown to mediate the impact of karrikins
490 in different developmental processes, including seed germination and root development
491 (Nelson *et al.*, 2011; Waters *et al.*, 2012). However, karrikins have been reported to have no
492 effect on shoot branching (Nelson *et al.*, 2011). It is therefore unlikely that the SL-independent
493 effect of D3/MAX2 on branching is dependent on karrikin signalling. Interestingly, MAX2 was
494 recently reported to be involved in CO_2 signalling in arabidopsis (Kalliola *et al.*, 2020). In
495 addition, new proteins interacting with MAX2 were recently discovered (Struk *et al.*, 2021),
496 showing that the regulation of MAX2 signalling is more complex than previously thought.
497 Altogether, these observations have demonstrated that D3/MAX2 is an important regulator
498 in the antagonistic regulation of shoot branching and tillering by sugars and strigolactones
499 (Figure 8).

500 The molecular mechanism through which sugars inhibit *D3/MAX2* expression is unknown.
501 However, recent studies have highlighted that sugars regulate shoot branching through
502 different sugar-signalling components such as Tre6P (Fichtner *et al.*, 2017, 2021a) and HXK1
503 (Barbier *et al.*, 2021). Interestingly, it was shown that *MAX2* expression negatively correlates
504 with *HXK1* expression and with sugar availability in arabidopsis (Barbier *et al.*, 2021).
505 Moreover, HXK1 deficiency leads to an upregulation of *MAX2* expression (Barbier *et al.*, 2021),
506 suggesting that the HXK1-signalling pathways may be involved in sugar regulation of *MAX2*
507 expression. However, HXK1 signalling is specific to glucose, and our previous data suggests
508 that a sucrose-specific signalling pathway was also involved in shoot branching (Barbier *et al.*,
509 2015b). Since Tre6P has been shown to be a sucrose-specific signal (Fichtner & Lunn, 2021),
510 it is reasonable to assume that this signalling component is also involved in the regulation of
511 *MAX2* expression by sugars. In addition, other sugar signalling pathways have been shown to
512 regulate *BRC1* expression via transcriptional (Wang *et al.*, 2021) and post-transcriptional

513 mechanisms (Wang *et al.*, 2019b). It is therefore possible that sugar-signalling mechanisms
514 independent of HXK1 and Tre6P may also be involved in the regulation of *MAX2* expression.
515 Future studies need to be done to understand how sugars regulate *MAX2* expression at the
516 molecular level.

517

518 **Sucrose prevents SL-induced D53 and D14 degradation to promote tillering**

519 The transcriptional inhibition of *D3/MAX2* by sucrose in diverse plants suggests that the effect
520 of sucrose on D53 may be mediated at least partly via *D3*. This hypothesis is strongly
521 supported by the evidence that sucrose is able to alleviate GR24-mediated D53 degradation
522 in a *D14* over-expressing line, but not in a *D3* over-expressing line (Figure 4 D, F). The inability
523 of sucrose to alleviate LUC-D53 protein degradation when co-transfected with the *35Sp:OsD3*
524 construct provides further supports that sucrose acts, at least partly, through D3 to regulate
525 D53 (Figures 4H and 8; Supplementary Figure S5).

526 In addition to degradation of D53 protein, D3 is also responsible for SL-mediated degradation
527 of D14 protein in rice (Hu *et al.*, 2017). We thus measured the effect of sucrose on HA-tagged
528 D14 protein levels. In the absence of sucrose, GR24 completely degraded the D14 protein
529 within 12 hrs, whereas in the presence of 4% sucrose, GR24 failed to degrade the D14 protein
530 (Figure 3E). This result is important for two reasons. Firstly, it shows that the effect of sucrose
531 on D53 protein levels could not be attributed to a negative effect of sucrose on D14 protein
532 levels. Secondly, it suggests that the effect of sucrose on D53 is likely to be mediated by D3
533 since protein accumulation pattern of D14 and D53 reflects what would be expected if *D3* was
534 down-regulated (Hu *et al.*, 2017) (Figure 8). Contrary to the HA-D14 protein, sucrose did not
535 show any positive or negative effect on HA-D3 protein levels, indicating sucrose acting
536 through transcriptional regulation of *D3* rather than through regulation of D3 protein stability
537 (Figure 3F).

538 Interestingly, we observed an over-accumulation of D53 protein in the *D14* over-expression
539 line (Figure 4A-D, Supplementary Figure S8), which further led to increased sucrose-induced
540 tillering in this line (Figure 5A-B). Considering the dual function of the D3 protein in
541 modulating both SL-induced D53 and D14 protein degradation (Chevalier *et al.*, 2014;

542 Soundappan *et al.*, 2015; Wang *et al.*, 2015; Hu *et al.*, 2017), it can be hypothesised that there
543 may be a competitive effect between D53 and D14 in binding to the D3 protein. Elevated D14
544 protein levels in the *D14* over-expressing line would recruit more D3 protein, creating a deficit
545 for D53 protein degradation. Whether and tillering remains to be determined. Interestingly,
546 in pea, D14 was reported to be a mobile protein between root and shoot (Kameoka *et al.*,
547 2016). In addition, D14 was reported to be highly abundant in the phloem in rice (Aki *et al.*,
548 2008) and arabidopsis (Batailler *et al.*, 2012). Combined with our data, this suggests that the
549 role of D14 in SL signalling is more complex than previously thought. More work is needed to
550 test whether sucrose acts directly on D14 to increase its protein levels and increase D53 levels
551 and to better understand the role of D14 in SL signalling.

552

553 **Sucrose also promotes tillering through a D3-independent pathway**

554 Our results also show that sucrose can increase D53 protein levels (Figure 4J) and inhibit *TB1*
555 expression (Supplementary Figure S6) in the *d3* background, without addition of exogenous
556 SL. In addition, bud elongation was also stimulated by sucrose in the *d3* background (Figure
557 6I-J), showing that at least one other pathway, independent of D3, is involved in the sucrose-
558 induced tillering. One straightforward explanation could be that the trophic properties of
559 sucrose also play a role in inducing D53 accumulation and tillering. However, other
560 hypotheses could also explain these observations. SL were reported to lead to the
561 degradation of D14 by the same process as that leading to D53 degradation (Hu *et al.*, 2017).
562 Our results also showed that sucrose prevents SL from degrading D14 (Figure 3E), which
563 makes senses if sucrose prevents the degradation of D53 by inhibiting *D3* (see section above).
564 This could also be explained by sucrose inhibiting the proteasome complex required for D3-
565 dependent D14 and D53 degradation. We also showed that increasing D14 led to higher levels
566 of D53 (Figure 4 A,D and Supplementary Figure S8). It could therefore be possible that sucrose
567 prevents the SL-induced D14 degradation to promote D53 accumulation (Figure 8). This could
568 be achieved by inhibiting the binding of SL to D14 or by preventing D3 from binding to D14.
569 Recent findings in pea suggested that CK promotes PsSMXL7 protein accumulation, the
570 homologue of the rice D53, through increasing the expression of *PsSMXL7* gene (Kerr *et al.*,
571 2021). Since sucrose was reported to promote CK synthesis in different species (Barbier *et al.*,

572 2015b; Kiba *et al.*, 2019; Salam *et al.*, 2021), it would be tempting to hypothesise that sucrose
573 induces D53 protein levels through a CK-mediated increase of *D53* expression. However, in
574 our conditions, sucrose did not promote *D53* expression, and even decreased its transcription
575 (Figure 3B), excluding this hypothesis.

576 Whether sucrose acts through D53 to regulate *TB1* expression independently of D3 remains
577 to be determined (Figure 3A and Supplementary Figure S6). As mentioned above, sugar
578 availability was reported to induce CK accumulation (Barbier *et al.*, 2015b; Kiba *et al.*, 2019;
579 Salam *et al.*, 2021), and CK were reported to inhibit *BRC1* expression (Dun *et al.*, 2012; Roman
580 *et al.*, 2016), which could explain our observations. It was also reported in rose calli, that
581 sucrose regulates *TB1/BRC1* expression through specific elements on the 3'UTR of this gene
582 (Wang *et al.*, 2019b), showing that sugars interact with SL signalling through multiple
583 pathways. More studies are needed to understand the complete mechanism underpinning
584 sugar-promoted tillering/branching and the interactions between SL and sugars.

585

586 **Conclusion**

587 The present study demonstrates that sugar availability and strigolactone signalling interact
588 during the control of shoot branching, and that D3 plays a role in this interaction but that
589 other pathways independent of D3 may also exist. In addition, our study shows that the
590 protein levels of D14 and D3 impact tillering in opposite ways, which should prompt further
591 studies to investigate the regulation of the components in the SL regulation of plant
592 development. SL and sugars are systemic signals and their levels in plants are tightly regulated
593 by environmental cues such as light, moisture and nutrient status (Yoneyama *et al.*, 2007;
594 Yoneyama *et al.*, 2012; Lemoine *et al.*, 2013; Kapulnik & Koltai, 2014). Their interactions
595 during the control of bud outgrowth therefore represents an important regulatory node in
596 the control of plant architecture in response to the environment. Our study therefore
597 provides an interesting opportunity to manipulate tillering and ultimately improve crop
598 management and yields through molecular engineering and crop selection. Nonetheless,
599 more work is needed to fully understand the mechanism(s) through which sucrose interact
600 with D14, D3 and D53, and to better understand the unexpected promoting role of D14 in
601 tillering.

602

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604

605 ## Supplementary Material:

606 **Supplementary Table S1.** Primers used in RT-qPCR analysis.

607 **Supplementary Figure S1.** Effect of sucrose and sorbitol on tillering.

608 **Supplementary Figure S2.** Map of the vector used for the D53 protein degradation
609 experiment performed on *Nicotiana benthamiana* leaves.

610 **Supplementary Figure S3.** Confirmation of gene expression of *OsD3* and *OsD14* in the
611 corresponding overexpressing transgenic lines.

612 **Supplementary Figure S4.** Phenotypes of transgenic plants constitutively expressed with *D3*
613 and *D14* under the control of *ACTIN1* promoter (ACTp).

614 **Supplementary Figure S5.** Effect of sucrose and GR24 on transiently expressed luciferase-D53
615 protein alone and co-transfected with 35S-OsD3 protein in *Nicotiana benthamiana* leaves.

616 **Supplementary Figure S6.** *OsTB1* expression in the rice calli treated with different
617 concentrations of sucrose for 24 hrs.

618 **Supplementary Figure S7.** Phenotypes of the *d3* and *d14* loss-of-function mutants in
619 comparison with WT in the field condition.

620 **Supplementary Figure S8.** Comparative analysis of D53 protein levels in different
621 backgrounds.

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810

811 ## Figures Legends:

812

813 **Figure 1. Sucrose alleviates the inhibitory effect of GR24 on tiller bud outgrowth in rice.** (**A**)
814 Representative tiller buds and (**B**) length of tiller buds of the WT (Huaidao 5) grown under
815 different sucrose concentrations with or without 1 µM GR24 for 3 weeks. Scale bar represents
816 10 mm. Different lower-case letters denote significant differences ($p<0.05$, one-way ANOVA
817 following Tukey's test for multiple comparisons). Error bars represent ± SE ($n > 8$).

818

819 **Figure 2. Sucrose alleviates the GR24-induced D53 degradation.** (**A**) Western blot showing
820 D53 protein accumulation in rice calli grown for 4 weeks on different sucrose concentrations.
821 (**B**) D53 protein degradation in the WT rice calli initially grown on NB plates containing 4%
822 sucrose and later shifted to liquid media with or without 4% sucrose containing 0.5 µM GR24
823 for different time points. (**C**) Transiently expressed luciferase-D53 protein in *Nicotiana*
824 *benthamiana* leaves subjected to 1 µM GR24 treatment with or without 4% sucrose for
825 different time points. Luciferase readings were normalised with Renilla luciferase readings.
826 Values are mean ± SE ($n = 4$). Different lower-case letters denote significant differences
827 ($p<0.05$, one-way ANOVA following Tukey's test for multiple comparisons) (**D**) Western blot
828 showing D53 accumulation in isolated tillers buds (< 3 mm length) of 3-month-old rice
829 (Nipponbare) plants treated with or without 4 % sucrose for 1 hour. Numbers in italics below
830 the blots indicate the relative band intensity of D53 normalized by the intensity of Actin or
831 HSP82 bands.

832

833 **Figure 3. Sucrose down-regulates key genes in the SL signalling pathway.** (**A**) Effect of
834 different sucrose concentrations on the expression of *TB1*, (**B**) *D53*, (**C**) *D14* and (**D**) *D3* in the
835 callus (grown for three weeks), shoot base tissues (three weeks old plants), and isolated tiller
836 buds (<3 mm length) of two-month-old rice plants grown hydroponically with different
837 sucrose concentrations for 24 hrs. Values are mean ± SE ($n = 3$). Each replicate consists of 8
838 biologically independent samples. Significant levels: * $p < 0.05$, ** $p < 0.01$; indicated by
839 Student's *t*-Test. (**E**) Effect of sucrose on GR24-mediated degradation of HA-tagged D14 fused

840 protein levels detected by immunoblotting with an anti-HA tag monoclonal antibody. (**F**)
841 Effect of sucrose on GR24-mediated degradation of HA-tagged D3 fused protein levels
842 detected by immunoblotting with an anti-HA tag monoclonal antibody. Numbers in italics
843 below the blots indicate the relative band intensity of D14 or D3 normalized by the intensity
844 of Actin or HSP82 bands.

845

846 **Figure 4. Over-expression of *D3*, but not *D14*, leads to D53 degradation in the presence of**
847 **sucrose.** (**A**) Effect of sucrose (4%) on D53 accumulation at different time points in WT and
848 *D14* over-expressing line, and (**B**) WT and *D3* over-expressing line detected by
849 immunoblotting with an anti-D53 polyclonal antibody. (**C**) Effect of sucrose on D53
850 degradation in the calli of WT (GSOR300192), (**D**) *D14* over-expressing line, (**E**) WT
851 (GSOR300002), and (**F**) *D3* over-expressing line initially grown on NB plates containing 4%
852 sucrose and later shifted to liquid media with or without 4% sucrose containing 0.5 µM GR24
853 for different time points detected by immunoblotting with an anti-D53 polyclonal antibody.
854 (**G**) Effect of sucrose on *Nicotiana benthamiana MAX2* (*NbMAX2*) expression in *Nicotiana*
855 *benthamiana* leaves infiltrated with or without sucrose solution (4%) at different time points.
856 Values are mean ± SE (n=3). (**H**) Transiently expressed luciferase-D53 protein alone and co-
857 transfected with 35S-OsD3 protein in *N. benthamiana* leaves subjected to 1 µM GR24
858 treatment with or without 4% sucrose for different time points. Luciferase readings were
859 normalised with renilla luciferase readings. Values are mean ± SE (n = 3). Significant levels:
860 **$p < 0.01$; indicated by Student's *t*-Test. (**I**) D53 accumulation in rice in WT and *d3* mutant
861 calli grown NB plates containing 4% sucrose and later shifted to liquid media with 0.5 µM
862 GR24 and a range of sucrose concentrations for 10 min. (**J**) D53 accumulation in rice in WT
863 and *d3* mutant calli continuously grown on different sucrose concentrations for 6 weeks
864 without exogenous GR24. Numbers in italics below the blots indicate the relative band
865 intensity of D53 normalized by the intensity of Actin or HSP82 bands.

866

867 **Figure 5. *D3* over-expression prevents sucrose from inhibiting the SL-induced tillering**
868 **suppression.** (**A**) Effect of sucrose on tiller bud elongation in the WT (GSOR300192), (**B**) *D14*
869 over-expression line, (**C**) WT (GSOR300002) and (**D**) *D3* over-expressing line grown with or

870 without 1 µM GR24 for 3 weeks. Values are mean ± SE (n = 10). Significant levels: ***p <
871 0.001; **p < 0.01, *p < 0.05; indicated by Student's *t*-Test.

872

873 **Figure 6. Growth of rice tiller buds in response to different sucrose concentrations.** (**A**)
874 Representative images of tiller buds of WT (Nipponbare), (**B**) SL signalling mutants *d14 (htd-*
875 *2)* and (**C**) *d3 (S2-215)* grown on different sucrose concentrations for three weeks. (Scale bar=
876 1 mm). Arrowhead represents tiller bud used for measurement. (**D**) Tiller bud outgrowth
877 (mm) in the WT (Nipponbare), *d14* (*htd-2*) and *d3* (*s2-215*) grown under the different sucrose
878 concentrations for three weeks. Different lower-case letters denote significant differences,
879 p<0.05, one-way ANOVA following Tukey's test for multiple comparisons (compared for each
880 sucrose concentration separately). Error bars represent ±SE (n > 8).

881

882 **Figure 7. Effect of sucrose and decapitation on bud elongation and expression of SL pathway**
883 **genes in pea buds.** (**A**) Effect of decapitation on gene expression of *PsD14,* (**B**) *PsD3 (RMS4)*
884 and (**C**) *BRC1* (*PsTB1*) at different time points in response to decapitation. Values are mean ±
885 SE (n = 3 pools of 20 buds). (**D**) Expression of SL signalling genes in axillary buds fed with
886 sucrose or sorbitol (osmotic control) through the petiole for 4h. Values are mean ± SE (n = 3
887 pools of 20 buds). Each replicate consists of 8 individual samples. Significant levels: ***p <
888 0.001; **p <0.01 indicated by Student's *t*-Test. (**E**) Length of single-node pea buds of WT
889 (Terese), *Psd14* (*rms3*) and *Psd3* (*rms4*) mutants grown *in vitro* with 30, 50 and 70 mM sucrose
890 for 6 days. Different lower-case letters denote significant differences at each concentration
891 (p<0.05, one-way ANOVA following Tukey's test for multiple comparisons). Error bars
892 represent ± SE (n = 8 individual buds).

893

894 **Figure 8. Proposed model of the interaction between sucrose and SL pathway.** In absence
895 of SL, D53 is not targeted by D14 and D3 and triggers tillering, although a residual SL-
896 independent effect of D3 on D53 may exist. In presence of SL, a complex is formed between
897 D14, D3 and D53, leading to the rapid degradation of D14 and D53, inhibiting tillering. In
898 presence of SL and sugars, sugars repress *D3*, thus preventing the SL-induced D14 and D53

899 degradation and triggering tillering. Sugars also prevent the SL-induced D53 degradation
900 through a D3-independent pathway and trigger a D14-dependent induction of D53
901 accumulation. The mechanism through which D14 promotes D53 accumulation remains
902 unknown (hypotheses are given in the discussion).

Figure 1

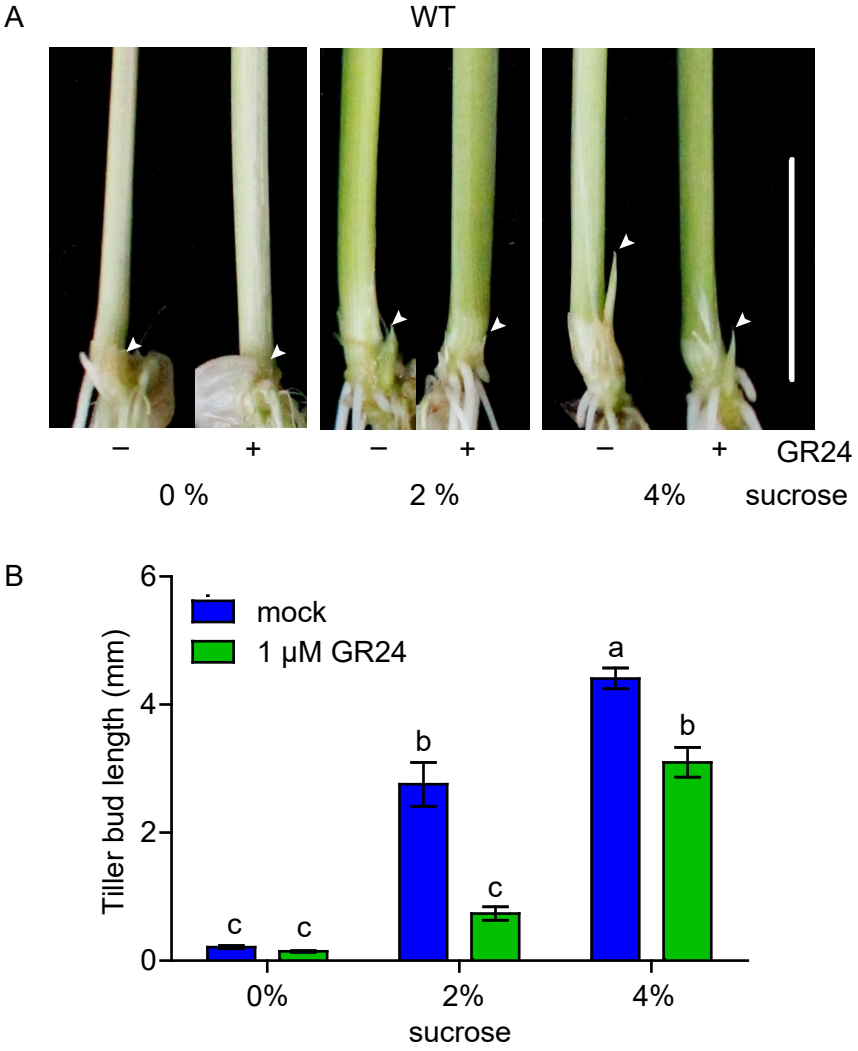

A
WT
–
+
–
+
–
+
GR24
0 %
2 %
4%
sucrose
B
mock
1 μM GR24
Tiller bud length (mm)
6
4
2
0
c
c
b
c
a
b
0%
2%
4%
sucrose

Figure 2

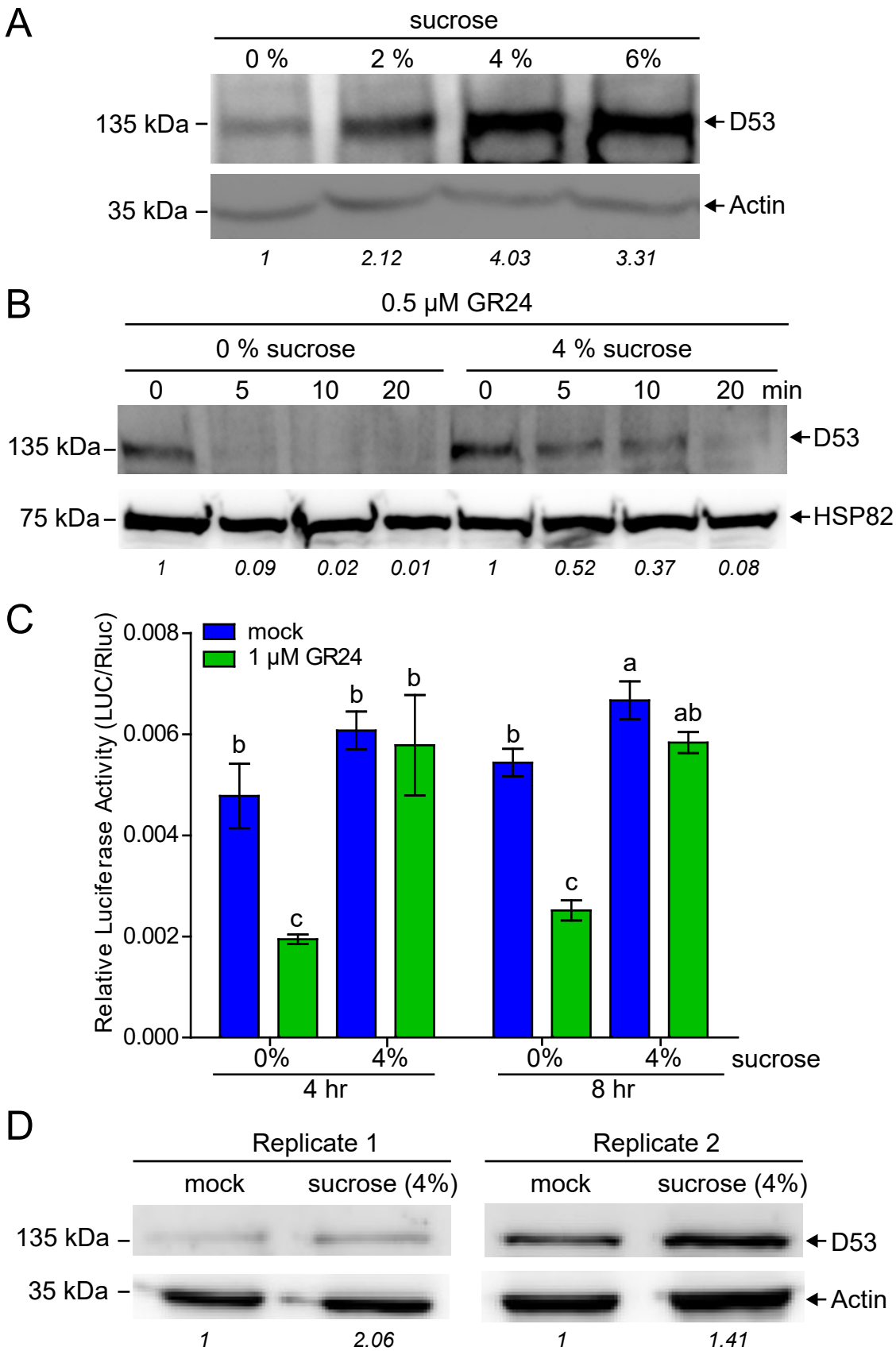

A
sucrose
0 %
2 %
4 %
6%
135 kDa
D53
35 kDa
Actin
1
2.12
4.03
3.31
B
0.5 μM GR24
0 % sucrose
4 % sucrose
0
5
10
20
0
5
10
20
min
135 kDa
D53
75 kDa
HSP82
1
0.09
0.02
0.01
1
0.52
0.37
0.08
C
Relative Luciferase Activity (LUC/Rluc)
0.008
0.006
0.004
0.002
0.000
mock
1 μM GR24
b
c
b
b
b
c
a
ab
0%
4%
0%
4%
sucrose
4 hr
8 hr
D
Replicate 1
Replicate 2
mock
sucrose (4%)
mock
sucrose (4%)
135 kDa
D53
35 kDa
Actin
1
2.06
1
1.41

Figure 3

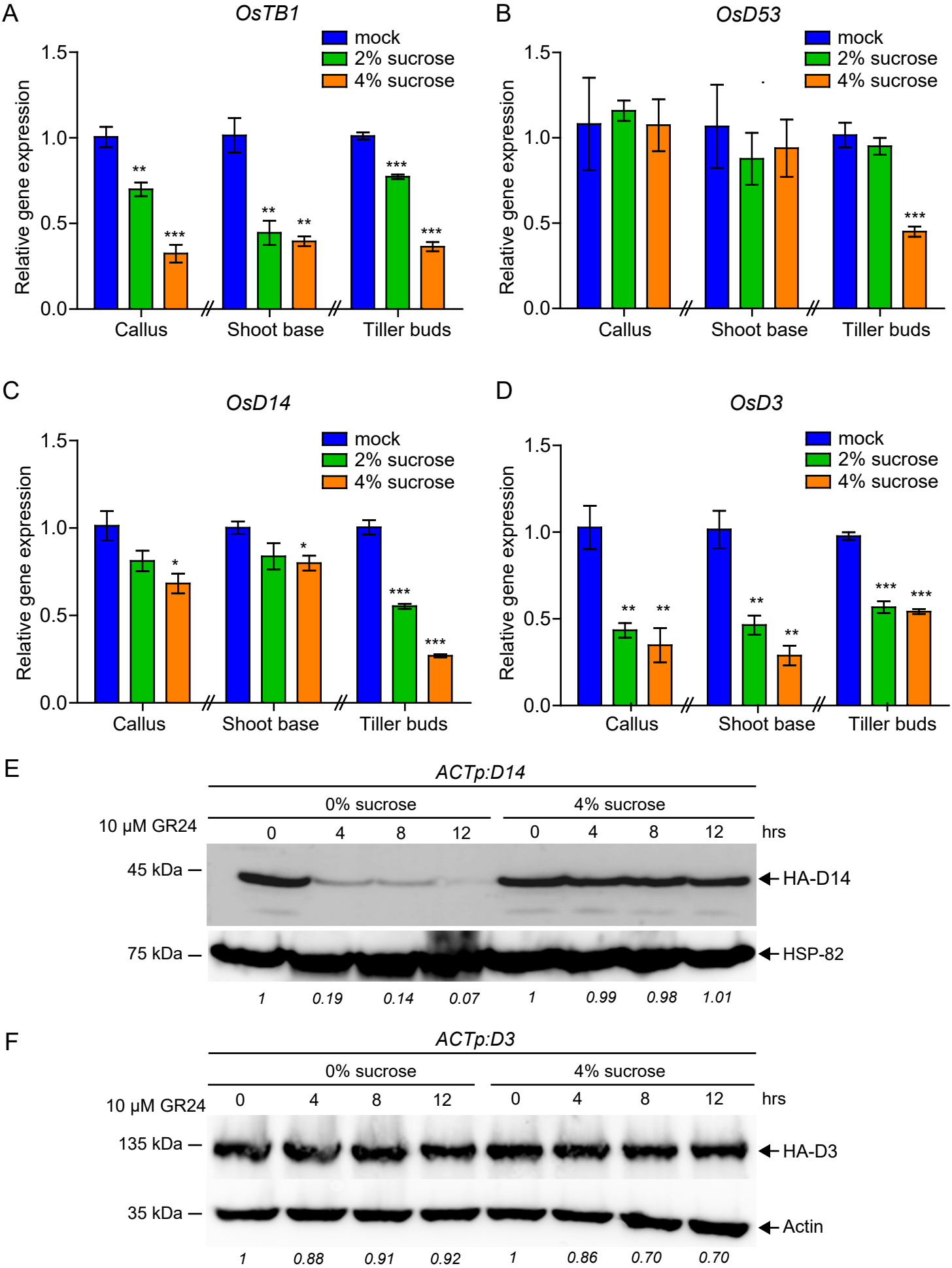

A
OsTB1
mock
2% sucrose
4% sucrose
Relative gene expression
1.5
1.0
0.5
0.0
**
***
**
**
***
***
Callus
Shoot base
Tiller buds
B
OsD53
mock
2% sucrose
4% sucrose
Relative gene expression
1.5
1.0
0.5
0.0
***
Callus
Shoot base
Tiller buds
C
OsD14
mock
2% sucrose
4% sucrose
Relative gene expression
1.5
1.0
0.5
0.0
*
*
***
***
Callus
Shoot base
Tiller buds
D
OsD3
mock
2% sucrose
4% sucrose
Relative gene expression
1.5
1.0
0.5
0.0
**
**
**
**
***
***
Callus
Shoot base
Tiller buds
E
ACTp:D14
0% sucrose
4% sucrose
10 μM GR24
0
4
8
12
0
4
8
12
hrs
45 kDa
HA-D14
75 kDa
HSP-82
1
0.19
0.14
0.07
1
0.99
0.98
1.01
F
ACTp:D3
0% sucrose
4% sucrose
10 μM GR24
0
4
8
12
0
4
8
12
hrs
135 kDa
HA-D3
35 kDa
Actin
1
0.88
0.91
0.92
1
0.86
0.70
0.70

Figure 4

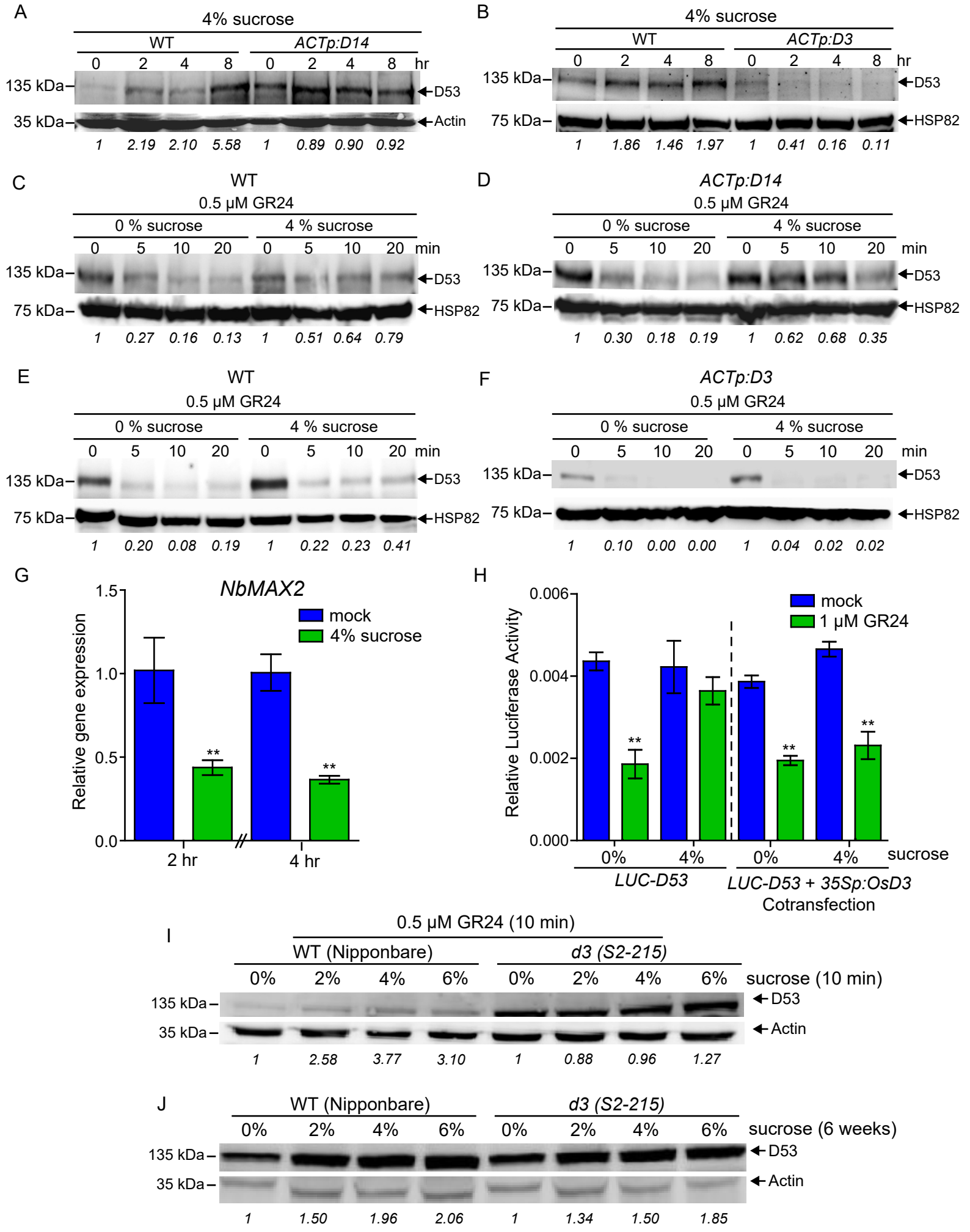

A
4% sucrose
WT
ACTp:D14
0 2 4 8 0 2 4 8 hr
135 kDa
35 kDa
D53
Actin
1 2.19 2.10 5.58 1 0.89 0.90 0.92
B
4% sucrose
WT
ACTp:D3
0 2 4 8 0 2 4 8 hr
135 kDa
75 kDa
D53
HSP82
1 1.86 1.46 1.97 1 0.41 0.16 0.11
C
WT
0.5 μM GR24
0 % sucrose
4 % sucrose
0 5 10 20 0 5 10 20 min
135 kDa
75 kDa
D53
HSP82
1 0.27 0.16 0.13 1 0.51 0.64 0.79
D
ACTp:D14
0.5 μM GR24
0 % sucrose
4 % sucrose
0 5 10 20 0 5 10 20 min
135 kDa
75 kDa
D53
HSP82
1 0.30 0.18 0.19 1 0.62 0.68 0.35
E
WT
0.5 μM GR24
0 % sucrose
4 % sucrose
0 5 10 20 0 5 10 20 min
135 kDa
75 kDa
D53
HSP82
1 0.20 0.08 0.19 1 0.22 0.23 0.41
F
ACTp:D3
0.5 μM GR24
0 % sucrose
4 % sucrose
0 5 10 20 0 5 10 20 min
135 kDa
75 kDa
D53
HSP82
1 0.10 0.00 0.00 1 0.04 0.02 0.02
G
NbMAX2
mock
4% sucrose
Relative gene expression
1.5
1.0
0.5
0.0
**
**
2 hr
4 hr
H
mock
1 μM GR24
Relative Luciferase Activity
0.006
0.004
0.002
0.000
**
**
**
0%
4%
0%
4%
sucrose
LUC-D53
LUC-D53 + 35Sp:OsD3
Cotransfection
I
0.5 μM GR24 (10 min)
WT (Nipponbare)
d3 (S2-215)
0% 2% 4% 6% 0% 2% 4% 6% sucrose (10 min)
135 kDa
35 kDa
D53
Actin
1 2.58 3.77 3.10 1 0.88 0.96 1.27
J
WT (Nipponbare)
d3 (S2-215)
0% 2% 4% 6% 0% 2% 4% 6% sucrose (6 weeks)
135 kDa
35 kDa
D53
Actin
1 1.50 1.96 2.06 1 1.34 1.50 1.85

Figure 5

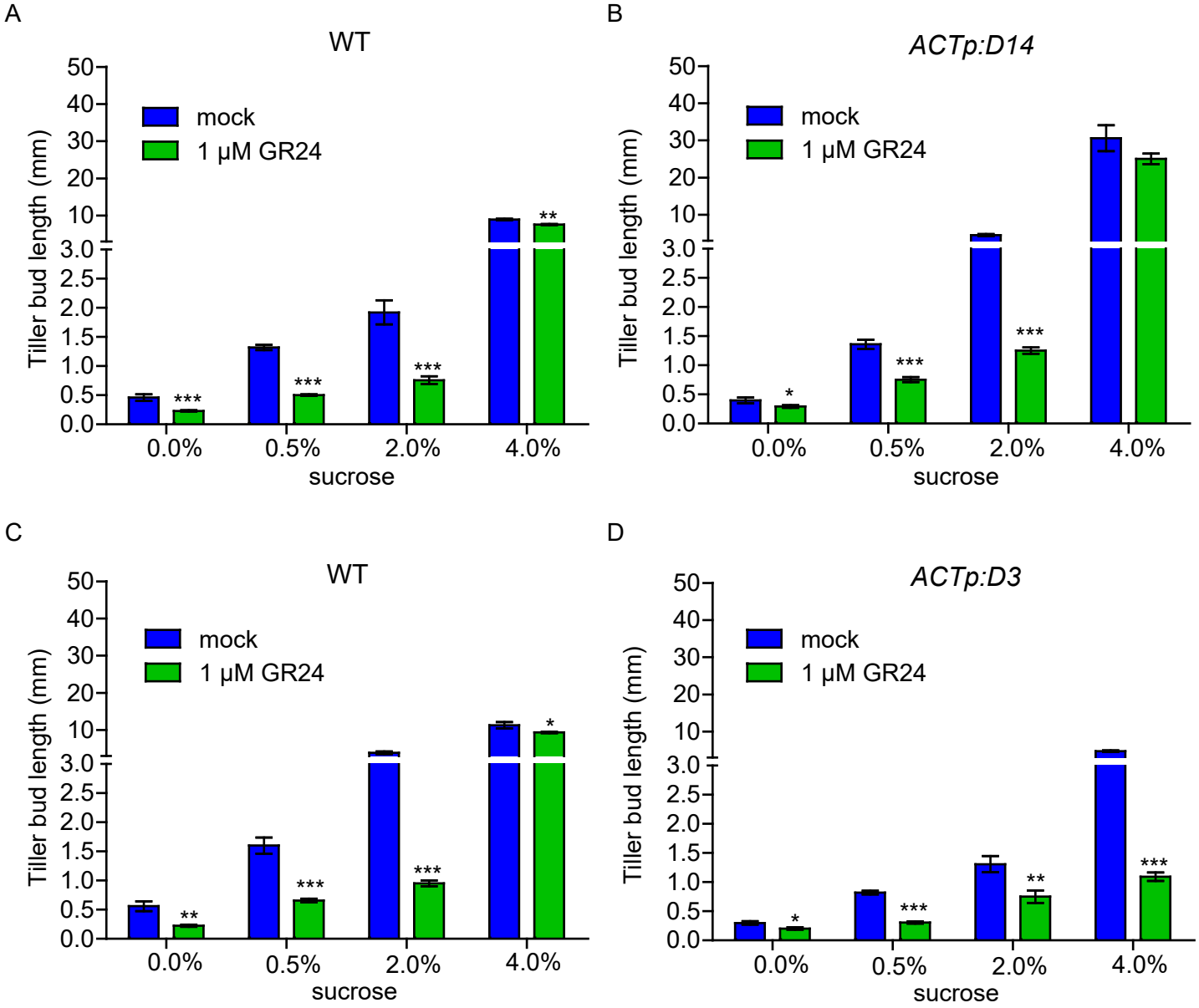

A
WT
mock
1 μM GR24
Tiller bud length (mm)
50
40
30
20
10
3.0
2.5
2.0
1.5
1.0
0.5
0.0
***
***
***
**
0.0%
0.5%
2.0%
4.0%
sucrose
B
ACTp:D14
mock
1 μM GR24
Tiller bud length (mm)
*
***
***
0.0%
0.5%
2.0%
4.0%
sucrose
C
WT
mock
1 μM GR24
Tiller bud length (mm)
**
***
***
*
0.0%
0.5%
2.0%
4.0%
sucrose
D
ACTp:D3
mock
1 μM GR24
Tiller bud length (mm)
*
***
**
***
0.0%
0.5%
2.0%
4.0%
sucrose

Figure 6

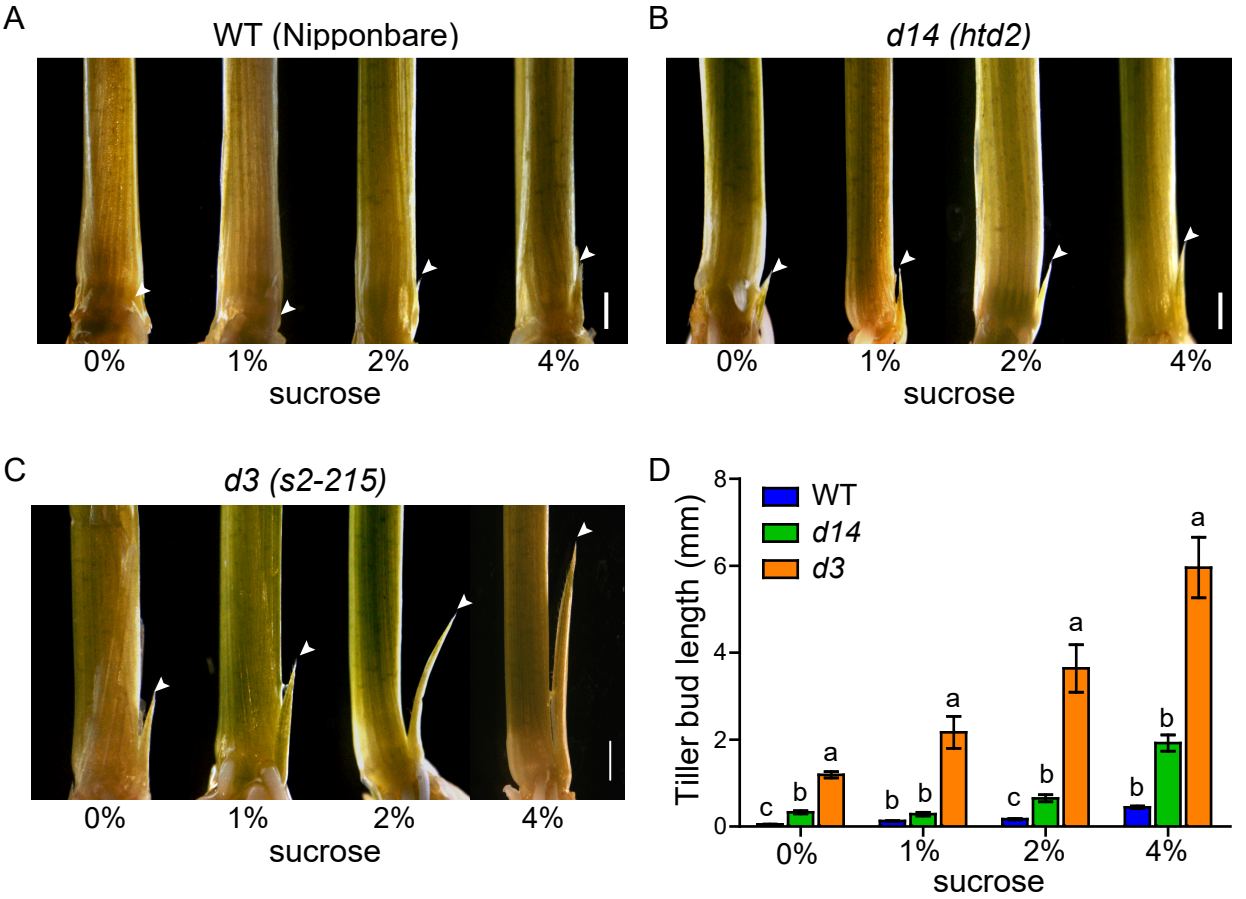

A
WT (Nipponbare)
0%
1%
2%
4%
sucrose
B
d14 (htd2)
0%
1%
2%
4%
sucrose
C
d3 (s2-215)
0%
1%
2%
4%
sucrose
D
WT
d14
d3
Tiller bud length (mm)
0
2
4
6
8
c
b
a
b
b
a
c
b
a
b
b
a
0%
1%
2%
4%
sucrose

Figure 7

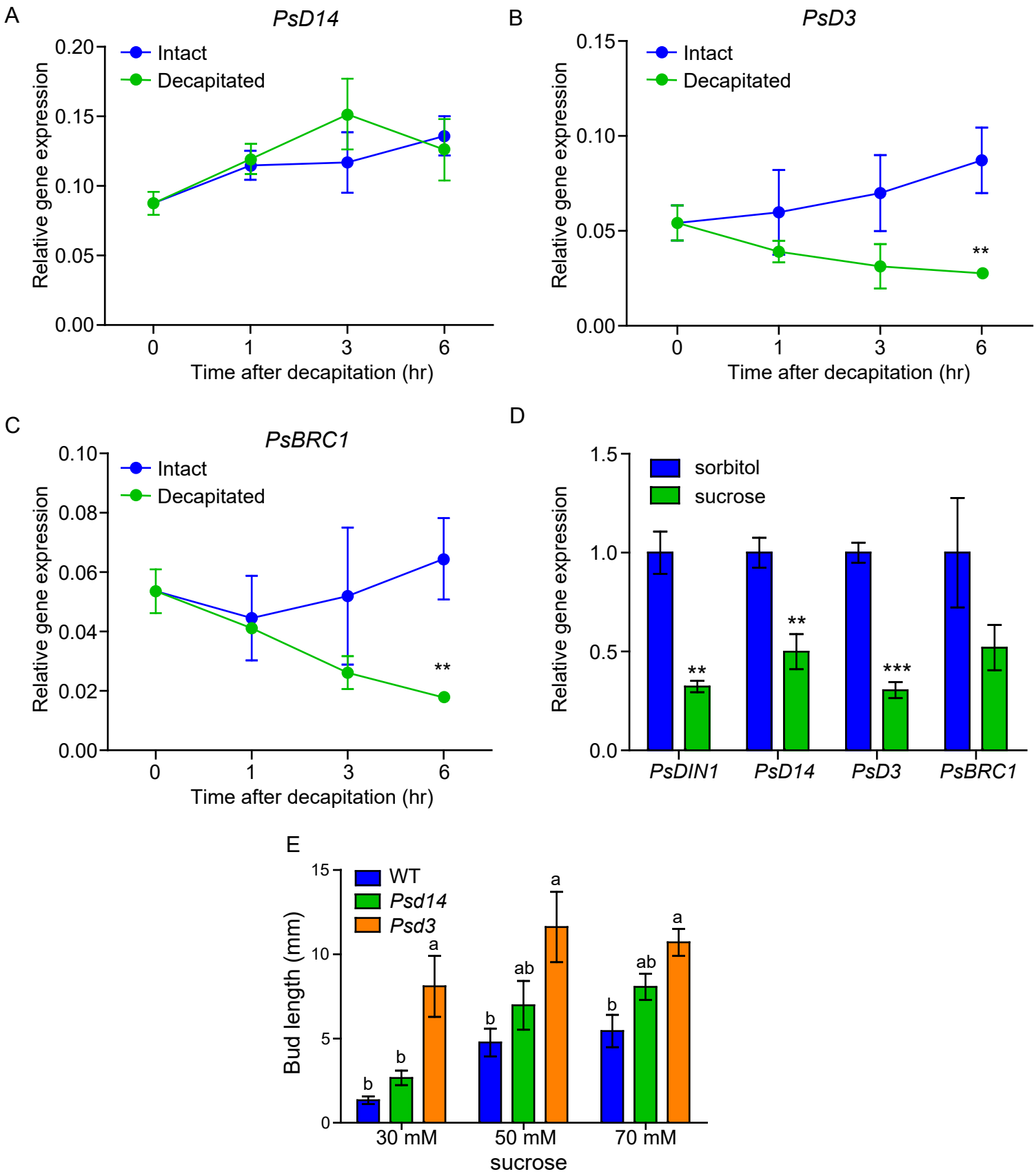
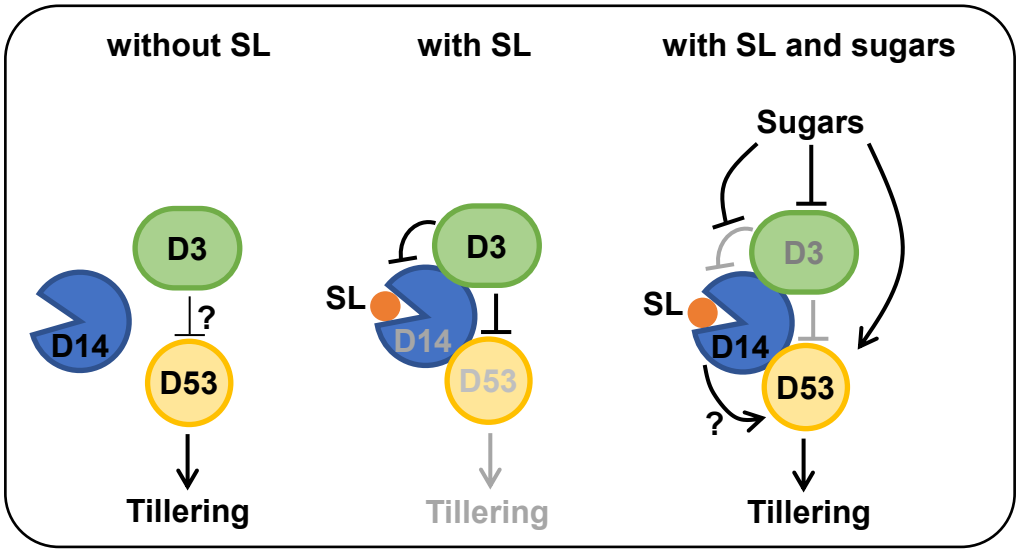

A
PsD14
Intact
Decapitated
Relative gene expression
0.20
0.15
0.10
0.05
0.00
0
1
3
6
Time after decapitation (hr)
B
PsD3
Intact
Decapitated
Relative gene expression
0.15
0.10
0.05
0.00
**
0
1
3
6
Time after decapitation (hr)
C
PsBRC1
Intact
Decapitated
Relative gene expression
0.10
0.08
0.06
0.04
0.02
0.00
**
0
1
3
6
Time after decapitation (hr)
D
sorbitol
sucrose
Relative gene expression
1.5
1.0
0.5
0.0
**
**
***
PsDIN1
PsD14
PsD3
PsBRC1
E
WT
Psd14
Psd3
Bud length (mm)
15
10
5
0
b
b
a
b
ab
a
b
ab
a
30 mM
50 mM
70 mM
sucrose

Figure 8

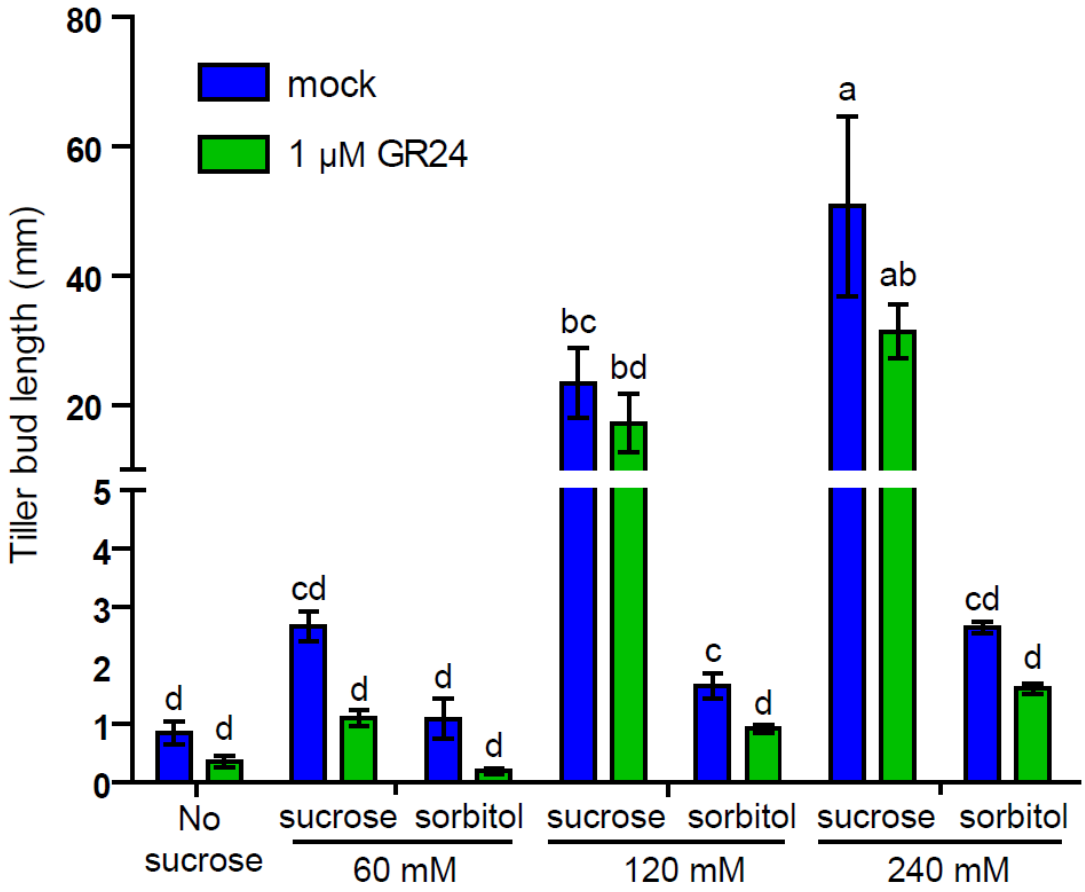

without SL
with SL
with SL and sugars
Sugars
D3
D14
?
D53
Tillering
SL
D3
D14
D53
Tillering
SL
D3
D14
D53
?
Tillering

Supplementary Table 1

Supplementary Table S1. Primers used in RT-qPCR analysis

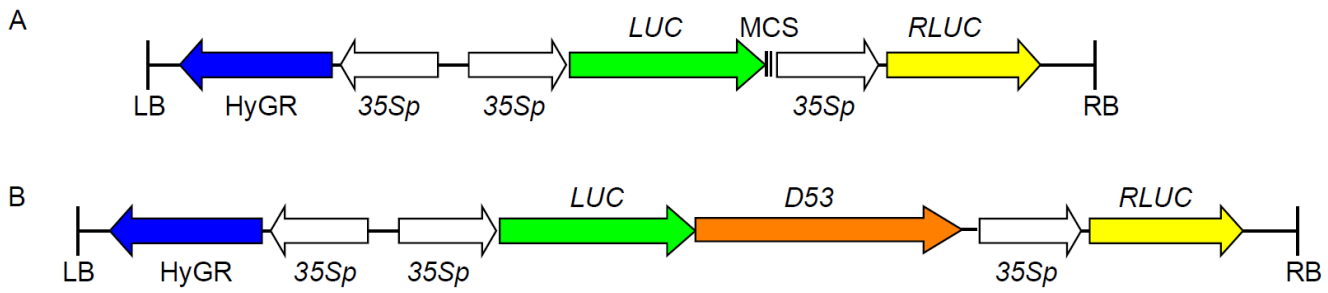
Sr. No	Gene	Forward primer (5'-3')	Reverse primer (5'-3')
1	*OsD3*	GGAACACCTCGACCTCTCGCTC	GAAGGCGTTCTGCTCGGAGATC
2	*OsD14*	GTGCTGTCGCATGGCTTC	GCAGGTCGTCGACGTAGG
3	*OsTB1*	CGACAGCGGCAGCTACTAC	GCGAATTGGCGTAGACGA
4	*OsD53*	CAACTATCATTAGCGCAAGTG	GTTGATCCTCTGGTGGTCTTGG
5	*NbMAX2*	TAATTGCAGGAGTGCGAGTG	TGGCATAGAAATGCCATGAA

Supplementary Figure S1



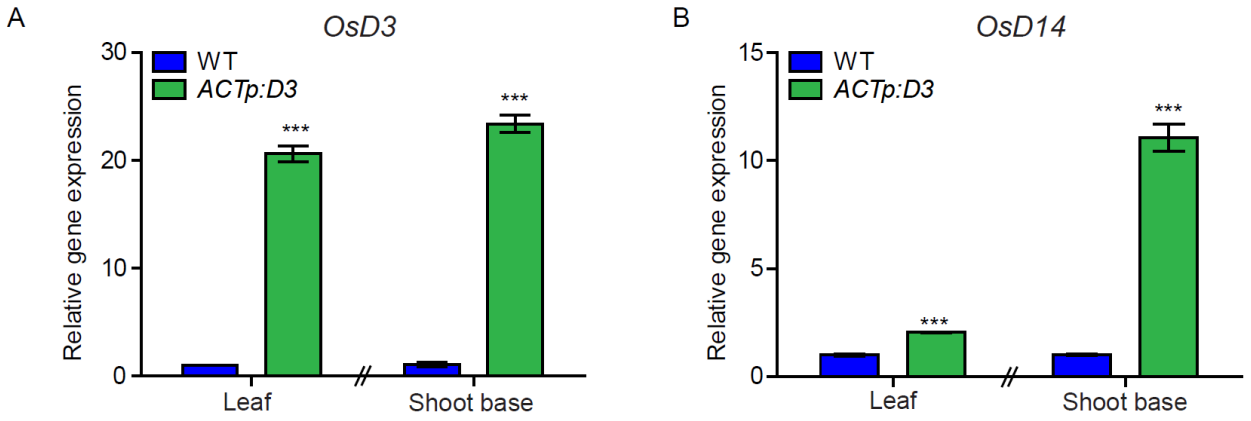
Supplementary Figure S1. Effect of sucrose and sorbitol on tillering. Length of tiller buds from rice plants fed hydroponically with different sucrose and sorbitol concentrations [No sucrose, 2% (~60 mM), 4% (~120 mM) and 8% (~240 mM)] with or without 1µM GR24. Different lower case letters denote significant differences, $p<0.05$, one-way ANOVA following Tukey's test for multiple comparisons. Error bars represent ± SE ($n > 8$).

Supplementary Figure S2



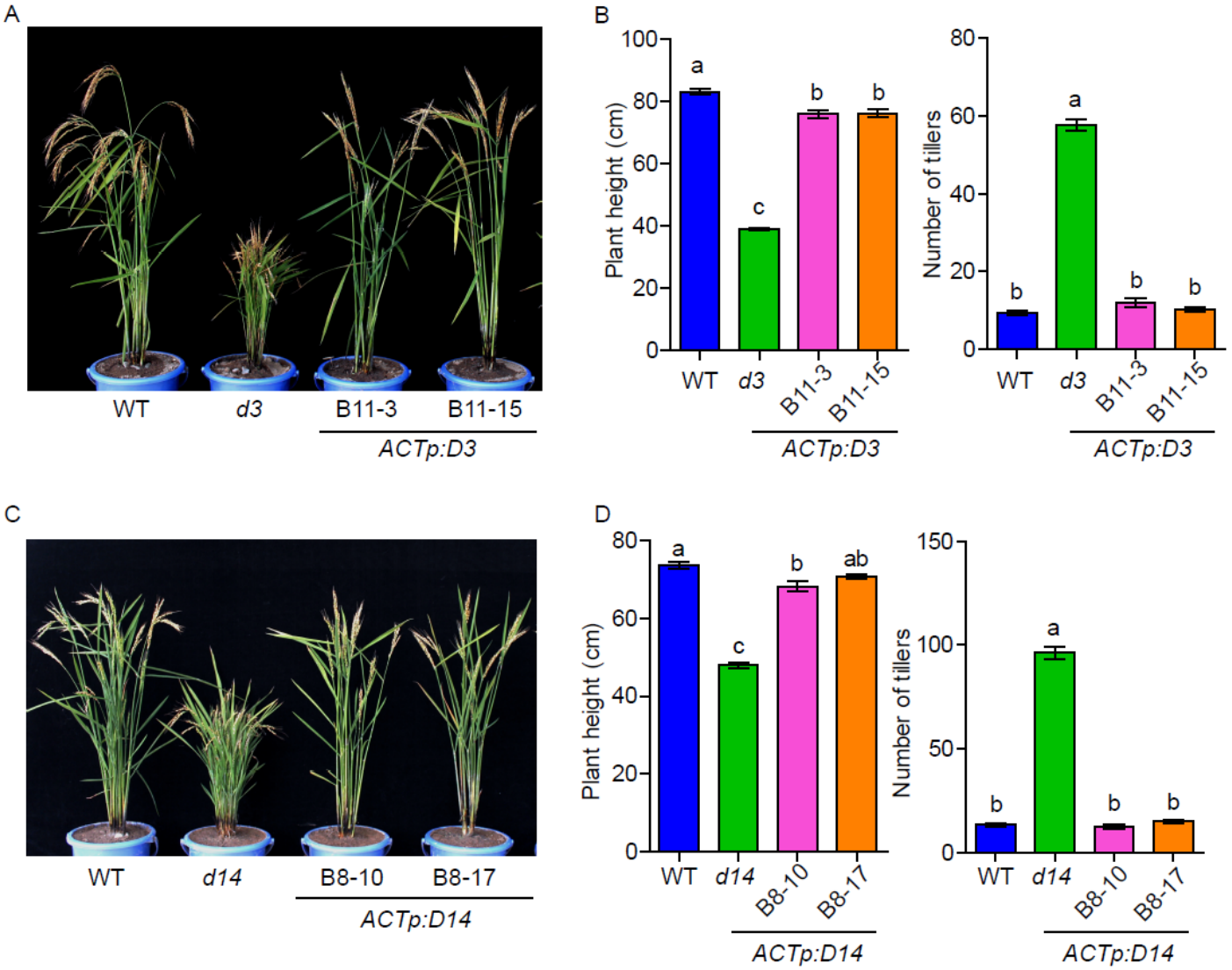
Supplementary Figure S2. Map of the vector used for the D53 protein degradation experiment performed in tobacco leaves. (**A**) Original vector (pCAMBIA 1200-R-LUC) consisting of the Fire fly luciferase and Renilla luciferase sequence driven by the CaMV 35S promoter. (**B**) The vector with the D53 protein fused at the C-terminal end of the Fire fly luciferase coding sequence driven by the CaMV 35S promoter in addition to the Renilla luciferase gene driven by the CaMV 35S promoter.

Supplementary Figure S3



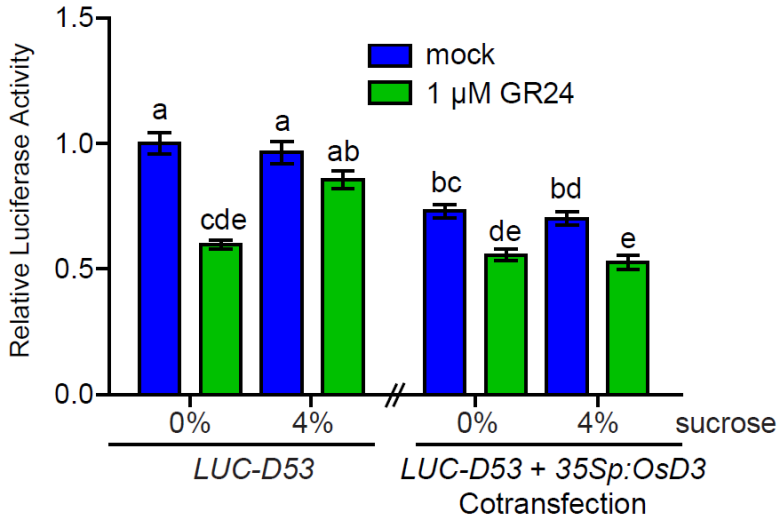
Supplementary Figure S3. Confirmation of gene expression of *OsD3* and *OsD14* in the corresponding overexpressing transgenic lines. Two-week-old rice seedlings were used to detect the expression of *OsD3* in the WT (GSOR300192), and *ACTp:D3* (*D3* over-expression line B11-15) and *OsD14* expression in the WT (GSOR300192) and *ACTp:D14* (*D14* over expression line B8-14). Significant levels (Compared to the WT), ***p <0.001 indicated by Student's t-Test.

Supplementary Figure S4



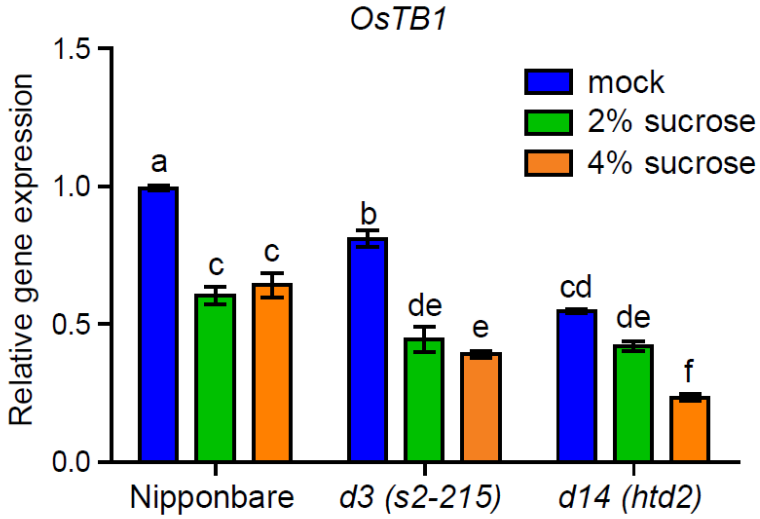
Supplementary Figure S4. Phenotypes of the d3 and d14 loss-of-function mutants in comparison with WT in the field condition.. (**A**) Phenotypes of WT (GSOR300192), *d3 (gsor300097)* and *ACTp:D3* transgenic plants at maturity stage in field condition. (**B**) Plant height (cm) and tiller numbers of the Phenotypes of WT (GSOR300192), *d3 (gsor300097)* and *ACTp:D3* transgenic lines at maturity stage in the field condition. Values are mean ±SE (n=20). (**C**) Phenotypes of WT (GSOR300192), *d14 (gsor300183)* and *ACTp:D14* transgenic plants at maturity stage in field condition. (**D**) Plant height (cm) and tiller numbers of the WT (GSOR300192), *d14 (gsor300183)* and *ACTp:D14* transgenic lines at maturity stage in the field condition. Different lower case letters denote significant differences ($p<0.05$, one-way ANOVA following Tukey's test for multiple comparisons). Error bars represent ± SE (n=20).

Supplementary Figure S5



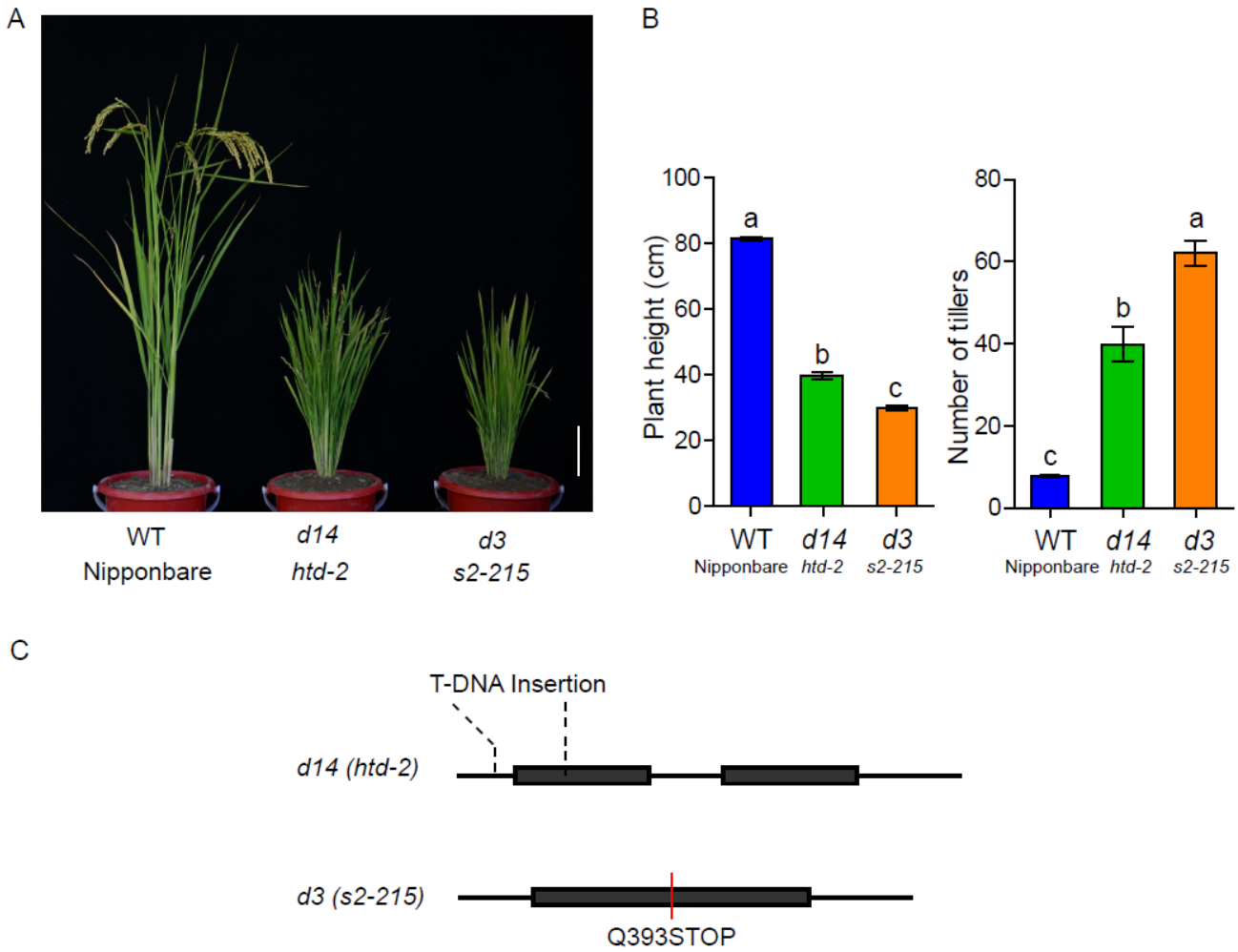
Supplementary Figure S5. Effect of sucrose and GR24 on transiently expressed luciferase-D53 protein alone and co-transfected with 35S-OsD3 protein in *Nicotiana benthamiana* leaves. Luciferase readings were normalised with renilla luciferase readings. Different lower-case letters denote significant differences ($p<0.05$, one-way ANOVA following Tukey's test for multiple comparisons). Error bars represent ± SE (n=6).

Supplementary Figure S6



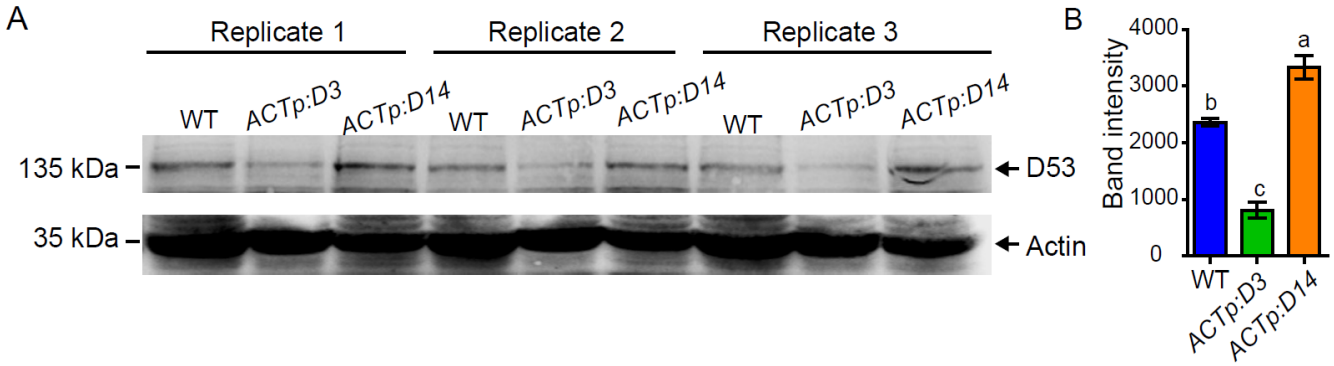
Supplementary Figure S6. *OsTB1* expression in the rice calli treated with different concentrations of sucrose for 24 hrs. Different lower-case letters denote significant differences ($p<0.05$, one-way ANOVA following Tukey's test for multiple comparisons). Error bars represent ± SE (n=6)).

Supplementary Figure S7



Supplementary Figure S7. Phenotypes of the *d3* and *d14* loss-of-function mutants in comparison with WT in the field condition. (**A**) Phenotypes of WT (Nipponbare), *d14 (htd-2)* and *d3 (s2-215)* mutants grown in the field condition. Scale bar=5 cm. (**B**) Plant height (cm) and tiller number of WT, *d14* and *d3* grown in the field condition. Different lower case letters denote significant differences ($p<0.05$, one-way ANOVA following Tukey's test for multiple comparisons). Error bars represent ± SE (n =8). (**C**) Mutation points of *d14 (htd2)* and *d3 (s2-215)* in the gene structure (black boxes represent exon and black line represent intron).

Supplementary Figure S8



Supplementary Figure S8. Comparative analysis of D53 protein levels in different backgrounds. (**A**) D53 protein levels in WT, *ACTp:D3* and *ACTp:D14* over-expressing lines grown on 4% sucrose plates detected by immunoblotting with an anti-D53 polyclonal antibody. (**B**) Average band intensity of the D53 proteins quantified in the WT, *ACTp:D3* and *ACTp:D14* over-expressing lines using Image J software. Values are mean ± SE (n=3), Different lower case letters denote significant differences, $p<0.05$, one-way ANOVA following Tukey's test for multiple comparisons.