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1 The *PROSCOOP10* gene encodes two extracellular hydroxylated peptides and 2 impacts flowering time in Arabidopsis

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28 introduction to the end of the acknowledgements, excluding materials and methods)

29 HIGHLIGHT

30 The *PROSCOOP10* gene encodes two post-translationally modified extracellular SCOOP10
31 peptides and acts upstream of *SOC1* and *LFY* to delay flowering.

32 RUNNING TITLE

33 Characterization of native SCOOP10 peptides and action of *PROSCOOP10* on floral transition

34 ABSTRACT

35 The Arabidopsis *PROSCOOP* genes belong to a family predicted to encode secreted pro-
36 peptides which undergo maturation steps to produce peptides named SCOOP. Some of them
37 are involved in defence signalling through their perception by a receptor complex including
38 MIK2, BAK1 and BKK1. Here, we focused on the *PROSCOOP10* gene which is highly and
39 constitutively expressed in the aerial organs. The MS/MS analyses of leaf apoplastic fluids
40 allowed the identification of two distinct peptides, named SCOOP10#1 and SCOOP10#2,

41 covering two different regions of PROSCOOP10. They both possess the canonical S-X-S
42 family motif and have hydroxylated prolines. This identification in apoplastic fluids confirms for
43 the first time the biological reality of SCOOP peptides. NMR and molecular dynamics studies
44 showed that the SCOOP10 peptides, although largely unstructured in solution, tend to assume
45 a hairpin-like fold exposing the two serine residues previously identified as essential for the
46 peptide activity. Furthermore, *PROSCOOP10* mutations led to an early flowering phenotype
47 and an increased expression of the floral integrators *SOC1* and *LEAFY*, consistent with the
48 transcription of *PROSCOOP10* in several mutants displaying an early or late flowering
49 phenotype. These results suggest a role of *PROSCOOP10* in flowering time, illustrating the
50 functional complexity of the *PROSCOOP* family.

51 **Keywords:** phyto cytokine, apoplasm, post-translational modification, flowering, Arabidopsis,
52 peptidomics

53 INTRODUCTION

54 Small secreted peptides originate from the processing of protein precursors that share a N-
55 terminal signal peptide addressing them to the secretory pathway. We distinguish (i) small
56 post-translationally modified peptides (PTMPs) that are produced by proteolytic processing
57 (Murphy *et al.*, 2012); and (ii) cysteine-rich peptides (CRPs) characterised by an even number
58 of cysteine residues involved in intramolecular disulfide bonds (Matsubayashi, 2011;
59 Tavormina *et al.*, 2015). An integrative approach combining bioinformatics, transcriptomics and
60 phenotyping has led to the identification of a gene family encoding precursors of putative small
61 PTMPs named Serine-riCh endOgenOus Peptides (SCOOPs) (Gully *et al.*, 2019). All the
62 predicted SCOOP peptides share a S-X-S motif and are specific to the *Brassicaceae* species.
63 Moreover, assays based on application of synthetic peptides have shown that SCOOP
64 peptides are phyto cytokines involved in defence signalling through their perception by the
65 MDIS1-INTERACTING leucine-rich repeat receptor kinase 2 (MIK2) (Hou *et al.*, 2021; Rhodes
66 *et al.*, 2021) and the two co-receptors BRI1-associated receptor kinase (BAK1) and BAK1-
67 LIKE 1 (BKK1) (Gully *et al.*, 2019; Hou *et al.*, 2021; Rhodes *et al.*, 2021). New studies tolerating
68 a greater variability in the C-terminal motif of the PROSCOOP amino acid sequence have
69 expanded the SCOOP family to 28 members (Zhang *et al.*, 2022). For example, the
70 SECRETED TRANSMEMBRANE PEPTIDE family (STMP) contains 10 members out of which
71 four were also annotated as SCOOP peptides: STMP1, STMP2, STMP8 and STMP10
72 correspond actually to SCOOP13, SCOOP14, SCOOP15 and SCOOP4 respectively (Yu *et al.*,
73 2019). Additionally, the ENHANCER OF VASCULAR WILT RESISTANCE 1 peptide
74 (EWR1) and four closely related peptides also encode functional SCOOP peptides (Zhang *et al.*,
75 2022). All these peptides share the SCOOP characteristics and can induce MIK2-

76 dependent immune responses. However, the transcription profiles of the *PROSCOOP* genes
77 are contrasted according to organs and various stimuli. This raises the question of the
78 involvement of the SCOOP peptides in different biological functions, notably in plant
79 development. Indeed, *PROSCOOP12* (*AT5G44585*) is constitutively expressed in roots and
80 recent studies have shown that SCOOP12 is a moderator of root elongation through the control
81 of reactive oxygen species (ROS) homeostasis (Guillou *et al.*, 2022). This study focuses on
82 *PROSCOOP10* (*AT5G44580*) which is highly expressed in the aerial parts. We show that
83 *PROSCOOP10* encodes a propeptide which gives rise to two distinct peptides that we have
84 identified in leaf apoplastic fluids. We identify a tendency of hairpin structure of these peptides
85 exposing the S-X-S SCOOP motif. Then, we also show an early flowering phenotype in
86 *proscop10* mutants suggesting a role in the development of the aerial part of the plant and
87 particularly in flowering time.

88

89 **MATERIALS AND METHODS**

90 **Plant material**

91 *Arabidopsis thaliana* ecotype Columbia (Col-0) was used as control. Two independent
92 *proscop10* mutant lines in the Col-0 background were used. A first line, named *proscop10-*
93 *1*, was a T-DNA insertion line obtained from NASC (SALK_059855C) and the primers used for
94 genotyping are listed in **Supplementary Table S1**. T-DNA insertion was checked by PCR on
95 *PROSCOOP10* and compared with PCR on *AtCOP1*, used as a PCR positive control, in Col-
96 0 and *proscop10-1* mutant line. The second line, named *proscop10-2*, was created using
97 the CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-
98 associated protein 9) method. We searched for *PROSCOOP10*-specific single guide RNA
99 (sgRNA) and checked possible off target sites in the *Arabidopsis* Col-0 genome using the
100 Crispor Tefor program (<http://crispor.tefor.net>). The 20-base long RNA guides with the
101 following sequences were used: 5'-GACCACGCTCCAGGCAGTAA-3' and 5'-
102 ATCAGGCAGTGGGCATGGTG-3' (**Supplementary Fig. S1A**). Vectors and methods to get
103 the CRISPR/Cas9 constructs were as in Charrier *et al.* (2019). *Arabidopsis* transformation was
104 applied as in Zang *et al.* (2006).

105 The soil-grown plants used for ROS assay and phenotyping were grown under long-day
106 conditions (16 h light at 22 °C/16 h dark at 21 °C, 70% relative humidity). Seedlings used for
107 seedling growth inhibition assay were grown under short-day conditions (8 h light at 22 °C/8 h
108 dark at 21 °C, 70% relative humidity). Plants used for mass spectrometry (MS) analysis were
109 cultivated on soil under short-day conditions (8 h light at 22 °C/16 h dark at 21 °C, 70% relative

110 humidity) during four weeks. Sodium and mercury vapor lights were used providing a light
111 intensity of $352.9 \mu\text{mol.m}^{-2}.\text{s}^{-1}$.

112 **Synthetic peptides**

113 The following peptides: flg22 (QRLSTGSRINSAKDDAAGLQIA), SCOOP12
114 (PVRSSQSSQAGGR), SCOOP10#1 (SAIGTOSSTSDHAOGSNG), SCOOP10#2
115 (GDIFTGOSGSGHGGGRTOAP) were obtained from GeneCust (Boynes, France), O
116 corresponding to hydroxyprolines. SCOOP10#2* (GDIFTGPSGSGHGGGRTPAP)
117 corresponding to SCOOP10#2 without hydroxyprolines was obtained from Eurogentec SA
118 (Seraing, Belgium). Peptides were synthesized with a minimum purification level of 95% and
119 diluted in water to the final concentration used for the assays. The SCOOP10#1 and
120 SCOOP10#2 peptide sequences were identical to the native sequences identified by mass
121 spectrometry.

122

123 **Mass Spectrometry (MS) analyses of extracellular fluids**

124 The extracellular fluids of rosettes were obtained according to Boudart *et al.* (2005) with slight
125 modifications. The buffer used for the vacuum infiltration contained 5 mM sodium acetate at
126 pH 4.6, with or without 0.3 M mannitol, and three protease inhibitors: 1 mM AEBSF
127 (ThermoFisher, Scientific, Rockford, IL, USA), 10 mM 1-10 phenanthroline (Sigma Aldrich
128 Chimie SARL, Saint-Quentin-Fallavier, France), and 100 μM E64 (Sigma-Aldrich). After
129 centrifugation at 200 *g* of the vacuum-infiltrated rosettes, the fluids were collected and
130 submitted to an ultrafiltration using an Amicon® Ultra 10K device — 10,000 NMWL (Merck
131 Chimie SAS, Darmstadt, Germany). The samples were then speedvac-dried prior to
132 solubilization in 10 mM DTT and 50 mM ammonium bicarbonate, followed by alkylation with
133 50 mM iodoacetamide. The samples were directly desalted by solid phase extraction (SPE) on
134 C18 cartridges (StrataTM-XL 8E-S043-TG, Phenomenex, Le Pecq, France) as described in
135 Balliau *et al.* (2018). Alternatively, the samples were subjected to tryptic digestion inside the
136 cartridge. The peptides were eluted with 70% acetonitrile/0.06% acetic acid prior to be
137 speedvac-dried. They were finally resuspended in 2% acetonitrile/0.1% formic acid. The
138 samples were analysed by MS with a Q ExactiveTM-Plus Hybrid Quadrupole-Orbitrap™ mass
139 spectrometer (Thermo-Fisher Scientific, Waltham, MA, USA) coupled with an Eksigent
140 NanoLC-Ultra® 2D HPLC (AB SCIEXTM, Redwood City, CA, USA) as described (Balliau *et*
141 *al.*, 2018), except for the chromatographic separation step that was shortened to 45 min.
142 Database search was performed as described (Duruflé *et al.*, 2019), except for the enzymatic
143 cleavage that was specified to “no enzyme”. Protein inference was performed using the

144 X!TandemPipeline (Langella *et al.*, 2017) with the following parameters: peptide E-value
145 smaller than 0.003, protein E-value smaller than 0.01, and one peptide per protein.

146

147 **Nuclear Magnetic Resonance (NMR) analyses**

148 SCOOP synthetic peptides were dissolved at concentrations between 0.5 mM and 1 mM in
149 both 50 mM phosphate buffer water solution, pH 6.6, containing 10% D₂O and in DMSO-d₆.
150 Deuterated sodium TSP-d₄ at a concentration of 100 μM was used as an internal reference
151 for chemical shift in aqueous buffers (Wishart *et al.*, 1995). Measurements were performed at
152 278 K for aqueous and at 298 K for DMSO samples. Almost complete assignment of amide
153 protons, non-exchanging protons and protonated ¹³C atoms was achieved in solution by
154 ¹H,¹³C-HSQC, ¹H,¹H-TOCSY (mixing time of 60 ms), and ¹H,¹H-NOESY (mixing time of 200
155 ms) recorded on a 500 MHz (11.74 T) Bruker spectrometer (Bruker France, Palaiseau)
156 equipped with a 5 mm BBI (Broadband Inverse) probe. TopSpin 4 (Bruker BioSpin) and
157 NMRFAM-SPARKY (Lee *et al.*, 2015) were used to process and to analyse NMR data.
158 Chemical shift deviations from random coil values were calculated using the “secondary
159 chemical shift analysis” option of NMRFAM-SPARKY (Lee *et al.*, 2015), a module based on
160 PACSY (Lee *et al.*, 2012).

161

162 **Molecular Dynamics (MD) simulations**

163 The starting structures for our peptides were obtained by I-Tasser (Yang *et al.*, 2015). *In silico*
164 mutagenesis was performed in CHIMERA using the Rotamers tool (Pettersen *et al.*, 2004).
165 The proline residues were replaced by hydroxyproline residues using YASARA (Krieger *et al.*,
166 2014). The GROMACS package v5.0.7 (Abraham *et al.*, 2015) was used to run MD
167 simulations. The AMBER99SB-ILDN (Lindorff-Larsen *et al.*, 2010) force field was used to
168 provide molecular mechanics parameters to our peptides. The peptides were put in a cubic
169 cell (“box”), the border of which is at least 1 nm from the protein, and we solvate it with TIP3P
170 explicit water molecules. Counterions were added, if necessary, to obtain a neutral system and
171 took the place of water molecules. Energy minimization and the temperature and pressure
172 equilibrations were done as described in Pokotylo *et al.*(2020). The equilibration time was also
173 set at 100 ps relaxation time. Once our peptide was well-equilibrated at the desired
174 temperature (300 K) and pressure (1 bar), we released the position restraints and run
175 production MD for data collection. The peptides were subjected to 500 ns simulation with 2 fs
176 time steps. To evaluate the reproducibility, the whole process (minimization, equilibration and
177 production run) was repeated thrice. PyMol (DeLano *et al.*, 2002) and VMD (Humphrey *et al.*,

178 1996) were used for visualisation. Graphs and images were created with GNUplot (Janert *et*
179 *al.*, 2010) and PyMol (DeLano *et al.*, 2002). All MD trajectories were analysed using
180 GROMACS tools (Smith *et al.*, 2010; Lemkul *et al.*, 2018) along the last 250 ns. Polar contacts
181 maps were determined by calculating the radial distribution function of each nitrogen and
182 oxygen atom from all others and taking its maximum intensity in the range of H-bonds and salt-
183 bridges. This allows to have an overview of all polar interatomic interactions and their
184 occurrence (Ramos-Martin *et al.*, 2020).

185

186 **Seedling growth inhibition assay**

187 Seedlings were germinated on MS (1X) agar (1%) and grown for five days before transferring
188 one seedling per well of 24-well plates containing 500 μ L of MS medium or MS medium
189 supplied with the indicated elicitor peptide to a final concentration of 1 μ M (six replicates per
190 elicitor peptide treatment). Fresh masses were measured and the experiment was repeated
191 thrice.

192

193 **ROS assay**

194 ROS production was determined by a luminol-based assay. Three five-week old seedlings
195 grown on MS plates were incubated in 200 μ L double distilled water (ddH₂O) overnight in a 1.5
196 mL centrifuge tube. Then, ddH₂O was replaced by 200 μ L of reaction solution containing 100
197 μ M of luminol and 10 μ g/mL of horseradish peroxidase (Sigma-Aldrich, Saint-Louis, USA)
198 supplemented with or without 1 μ M peptide. Luminescence was measured for 60 min with a
199 one-second interval, immediately after adding the solution with a FLUOstar OPTIMA plate
200 reader (BMG LABTECH, Ortenberg, Germany). The total values of ROS production were
201 indicated as means of the relative light units (RLUs).

202

203 **Gene expression analysis**

204 For each of the three biological repetitions, shoot apical meristem (SAMs) samples were hand-
205 dissected under binocular magnifier, trying to remove as much leaf tissue as possible, from 15
206 individual 7 and 11-day old plants of Col-0, *proscop10-1* and *proscop10-2*, growing under
207 long-day conditions. Total RNAs were extracted using the Nucleospin RNA Plus Kit
208 (Macherey-Nagel, Düren, Germany). cDNAs were synthesized from 1.5 μ g of total RNA with
209 oligo(dT) primers using Moloney Murine Leukemia Virus Reverse Transcriptase MMLV-RT
210 according to the manufacturer's instructions (Promega, Madison, WI, USA). RT-qPCR was

211 carried out in a Chromo4 system (Bio-Rad, Laboratories, CA, USA). Expression profiles of key
212 floral transition genes were calculated using the $2^{-\Delta\Delta Ct}$ method and were corrected as
213 recommended in Vandesompele *et al.* (2002), with three internal reference genes (*ACT2*,
214 *COP1* and *AP4M*) used for the calculation of a normalization factor. Mean expression level of
215 Col-0 at 7 days served as calibrator. Primers for RT-qPCR analysis used are specified in
216 **Supplementary Table S1**.

217

218 **RESULTS**

219 **Identification of SCOOP10 peptides in extracellular fluids by MS**

220 Based on the high expression level of *PROSCOOP10* in leaves, we used a proteomic
221 approach to explore the apoplastic fluid content and search for the native form(s) of SCOOP10.
222 The experiment has been repeated thrice, twice without mannitol in the infiltration buffer and
223 once in the presence of 0.3 M mannitol. The results were similar. In one case, a tryptic digestion
224 of the proteins has been performed prior to the MS analysis. Different peptides could be
225 identified covering two different regions of PROSCOOP10: they are indicated as SCOOP10#1
226 and SCOOP10#2 in **Fig. 1**. The corresponding MS/MS spectra are shown in **Supplementary**
227 **Fig. S2**. The native SCOOP10#1 peptide comprises 18 amino acids and it covers the central
228 predicted conserved motif defined after the comparison of the amino acid sequences of the
229 members of the PROSCOOP family (Gully *et al.*, 2019): SAIGTPSSTSDHAPGSNG (**Fig. 1A,**
230 **B**). It contains the two strictly conserved serine (S) residues. All the observed peptides are
231 native ones, as shown by the absence of tryptic sites at their N- and C-termini. SCOOP10#1
232 was only observed thrice compared to the 38 observations of SCOOP10#2. In all these three
233 occurrences, it contained two hydroxyproline (O) residues. The native SCOOP10#2 peptide
234 comprises 20 amino acids at the most and covers the C-terminal predicted conserved motif of
235 the PROSCOOP family (Gully *et al.*, 2019): GDIFTGPSGSGHGGGRTAP (**Fig. 1A, C**). As
236 SCOOP10#1, it also contains the two strictly conserved serine residues. It also carries three
237 well-conserved successive glycine (G) residues. The C-terminus observed at arginine (R)16
238 in ten cases resulted from tryptic digestion and the corresponding peptides should not be
239 considered as native ones. All the other observed peptides are native ones because they are
240 not surrounded by tryptic sites. Contrary to SCOOP10#1, the pattern of proline (P)
241 hydroxylation is variable: We could observe hydroxyproline residues at either of the three
242 possible positions (P7, P18 or P20) and in various combinations (**Fig. 1B**). The most frequently
243 observed position of P hydroxylation was P18 (65.8%), followed by P7 (36.8%). In some cases,
244 P hydroxylation was observed at two positions on the same peptide: P7 and P18 (23.7%) or
245 P7 and P20 (7.9%). The C-terminus of the peptide was variable ending with P18, alanine A19

246 or P20. Although protease inhibitors are used already at the beginning of the experiment, the
247 variability of the C-terminus could be due to proteolysis by serine carboxypeptidases identified
248 in cell wall proteomes (see *WallProtDB-2*, San Clemente and Jamet, 2015; San Clemente *et*
249 *al.*, 2022).

250

251 **SCOOP10#2 is unstructured in solution but cis-trans isomerization may be favoured in** 252 **a hydrophobic environment**

253 According to MS analyses, SCOOP10#2 seems to be the major secreted or the more stable
254 peptide produced by *PROSCOOP10*. Therefore, now knowing its exact sequence, we decided
255 to study its structural behaviour in solution, using a hydroxylated synthetic SCOOP10#2
256 peptide (GDIFTGOSGSGHGGGRTOAP) and a non-hydroxylated one, SCOOP10#2*
257 (GDIFTGPSGSGHGGGRTPAP).

258 NMR data indicated that the synthetic SCOOP10#2 peptide is largely unstructured in water
259 solution. The NMR assignment of both non-hydroxylated (SCOOP10#2*) and hydroxylated
260 (SCOOP10#2) forms are reported in **Supplementary Tables S2 and S3**, respectively. The
261 chemical shift of some nuclei, namely H α , C α , C β and carbonyl, can be used to ascertain the
262 presence of secondary structure by observing their deviations from random coil values
263 (Wishart *et al.*, 1992; Wishart *et al.*, 1994; Wishart *et al.*, 2011) which can be predicted by the
264 peptide sequence (Nielsen *et al.*, 2018). The H α /C α region of the ^1H , ^{13}C -HSQC NMR spectrum
265 is shown in **Fig. 2A**, while the chemical shift deviations from random coil values are reported
266 in **Fig. 2B**. The presence of alpha helical structure can be monitored by at least three
267 consecutive negative H α , C β or positive C α deviations while the opposite holds for beta
268 strands. The difference between C α and C β deviations is often used as a combined predictor.
269 Overall, the observed deviations are below the threshold value of 0.1 ppm for ^1H and 0.7 ppm
270 for ^{13}C (Wang *et al.*, 2002). Hydroxylation of P7 and P18 has no major impact on the structure
271 as indicated by minimal chemical shift perturbations on most resonances (**Fig. 2A**). Large
272 changes are limited to the atoms of hydroxyprolines (O) and particularly to C γ carbon atoms
273 as expected.

274 NMR spectra also allowed monitoring the conformation of the peptide bond linking proline
275 residues to the previous amino acid (Dorman *et al.*, 1973; Siemon *et al.*, 1975). Such bond can
276 often assume *cis* conformation whereas it is commonly found *trans* in all other amino acid
277 types (Wedemeyer *et al.*, 2002). A *cis* conformation determines a rather radical change in the
278 direction of the peptide backbone and might be crucial for the biological function. In particular,
279 the H α of the preceding residue is close in space to the H α or H δ protons of proline in the *cis*
280 and *trans* conformation, respectively. Its resonance can therefore be used to identify each

281 conformer in NOESY or ROESY spectra (Gaggelli *et al.*, 2001). A clear NOESY peak between
282 the H α protons of G6 and H δ protons of P7 (or O7) revealed that the peptide bond is mainly in
283 *trans* conformation.

284 In order to investigate the structural behaviour of SCOOP10#2 in a more apolar environment
285 which could mimic that of its receptor, we studied the peptide in DMSO (**Supplementary Fig.**
286 **S3 and Supplementary Tables S4 and S5**). Interestingly, we observed a doubling of peaks
287 arising from residues 6-10, which is the region comprising the two conserved serine residues
288 (**Fig. 1A**). Analysis of NOESY spectra reveals that the minor form might belong to the *cis*
289 conformation of the G6-P7 peptide bond, as a cross peak is present between the H α proton of
290 G6 and an H α compatible with a second form of P7. The same was not observed for P18 and
291 P20.

292

293 **Transient hairpin-like structures exposing S8 and S10 might have functional relevance**

294 Molecular dynamics simulations also pointed at the absence of a well-defined structure in both
295 SCOOP10#2* and its hydroxylated form, in agreement with NMR data. Indeed, during MD
296 simulations, an ensemble of different conformations continuously interconverted along the
297 trajectory (**Fig. 3A**). However, these data revealed a certain tendency of the backbone to fold
298 at the level of residues O7-S10, thus exposing S8 and S10 side chains. Interestingly, this
299 region is the one displaying negative H α and positive C α -C β deviations in NMR, as expected
300 for the turn of a helix (**Fig. 3B**). Although below the threshold values, these data suggest the
301 presence of a small population of conformers contributing to the average chemical shift value.
302 For this reason, we analysed molecular dynamic trajectories, in order to reveal key interatomic-
303 interactions that might stabilise such a fold. For SCOOP10#2, the obtained polar contacts map
304 reveals the formation of an H-bond between the amide nitrogen of serine S10 and the carbonyl
305 of hydroxyproline O7 (**Fig. 3C**) or proline P7 (data not shown), a salt bridge between the side
306 chains of aspartic acid D2 and arginine R16, and a salt bridge between the C- and N-termini
307 (**Fig. 3D**). Furthermore, a stacking between the aromatic rings of phenylalanine F4 and
308 histidine H12 might also further stabilise the conformation (**Fig. 3D**). Similar contacts are
309 observed in the non-hydroxylated form (data not shown).

310 In order to test the relative contribution of each of these key inter-residue interactions we
311 performed multiple molecular dynamic simulations where we mutated at least one partner
312 residues: R16A, G13,14,15A, P7A and S8,10A (**Supplementary Figure S4A**). In the R16A
313 mutant, where we eliminated the salt bridge between D2 and R16, SCOOP10#2* still tends to
314 fold onto itself but at the level of residues 12-15 (**Supplementary Figure S4B**), thus reducing
315 the exposition of S8 (**Supplementary Figure S5**). In this case the driving force is the salt

316 bridge between the N and C termini. When glycine residues in the flexible region G13,14,15
317 are mutated to alanine, the N-C terminal interaction favours a fold in the middle of the structure
318 (residues 9-10) (**Supplementary Fig. S4B**). As for the H-bond between the amide of S10 and
319 P7, mutants (P7A and double mutant S8,10A) reveal small perturbation of the structural
320 behaviour (in P7A the structure folds around residue 8 by a turn rather than a helix 3-10), an
321 effect somehow expected considering that the interaction is established at the level of the
322 backbone.

323

324 **SCOOP10#1 is unstructured in solution with transient head-to-tail contact**

325 We also studied the structural behaviour in solution of SCOOP10#1, using a synthetic
326 SCOOP10#1 peptide identical to the native form identified (SAIGTOSSTSDHAOGSNG). For
327 the synthetic SCOOP10#1 peptide, deviations from random coil values also indicate a poor
328 structuring (**Supplementary Fig. S6**, and the NMR assignment in different conditions in
329 **Supplementary Tables S6 and S7**), and NOESY spectra are compatible with *trans*
330 conformation for the peptide bonds involving both P6 and P14. Contrarily to what observed for
331 SCOOP10#2, we could not detect the presence of *cis* conformation in the more apolar
332 environment of DMSO. MD simulations (**Supplementary Fig. S7**) agree with NMR data and
333 detect lack of structure (**Supplementary Fig. S7A**), however, infrequent contacts between the
334 N- and C-termini (**Supplementary Fig. S7B**) generate folded conformations vaguely
335 resembling those observed for SCOOP10#2 (see contact map in **Supplementary. Fig. S7C**).

336

337 **SCOOP10 peptides do not induce ROS production and growth inhibition**

338 Effects of the SCOOP10 synthetic peptides on seedling growth and ROS production were
339 tested on Col-0 and *mik2-1* genotypes and compared to those of the SCOOP12 peptide, as a
340 control (**Fig. 4**). The SCOOP12 amino acid sequence was the same already used by Gully *et*
341 *al.* (2019) and Guillou *et al.* (2022) and based on prediction without post-translational
342 modifications.

343 In Col-0 background and at 1 μ M, we showed that, the SCOOP10#2* peptide induced a
344 seedling growth inhibition which was much smaller than that induced by the SCOOP12 peptide.
345 In the same conditions, the SCOOP10#1 and SCOOP10#2 peptides did not show a significant
346 effect. In the *mik2-1* mutant background, none of the SCOOP peptides had an effect on growth,
347 as expected (**Fig. 4A**). At the same concentration, the SCOOP12 peptide induced a strong
348 ROS production in Col-0 leaves, as already reported (Gully *et al.*, 2019; Rhodes *et al.*, 2021).
349 On the contrary, neither the SCOOP10#2* nor the SCOOP10#1 and the SCOOP10#2

350 peptides, even simultaneously applied, induce ROS production in leaves. As expected none
351 of the SCOOP peptides induced a high ROS production in the *mik2-1* mutant (**Fig. 4B**).

352

353 **Mutations of *PROSCOOP10* impact flowering time**

354 Previous analysis of transcriptomic data showed that *PROSCOOP10* is highly expressed in
355 shoot apex and leaves and may play a role in aerial organ development (Gully *et al.*, 2019;
356 Hou *et al.*, 2021). Therefore, we compared the leaf development and flowering time of wild-
357 type and *proscop10* plants. The first *proscop10* mutant (*proscop10-1*) is a T-DNA insertion
358 line ordered from NASC (**Supplementary Fig. S1A**). This line was genotyped as mutated on
359 both DNA and cDNA (**Supplementary Fig. S1B**). We generated a second line (*proscop10-*
360 *2*) using a CRISPR/Cas9 approach and the mutant was genotyped (**Supplementary Fig. S1C,**
361 **D**). *proscop10-2* was a bi allelic mutant, which is frequently observed with this approach
362 (Pauwels *et al.*, 2018). In the first modified allele, a deletion of 237 bp occurred between the
363 two guides in addition to modifications of nucleotides within the guides, leading to a frameshift.
364 For the second modified allele, a deletion of 448 bp occurred between the two guides
365 (**Supplementary Fig. S1E**). In each case, the modifications prevented the synthesis of the
366 native SCOOP10 peptides (**Supplementary Fig. S1F**).

367 Both mutant lines displayed a normal vegetative development but 22 days after germination,
368 the inflorescences of *proscop10* mutants bolted significantly earlier than those of Col-0 plants,
369 with a shift of two days (**Fig. 5A, C**) and the number of rosette leaves were significantly lower
370 for *proscop10* at bolting day (**Fig. 5E**). Consequently, five days after, mutants had longer
371 stem length compared to Col-0 (**Fig. 5B**). 80% of the inflorescence of *proscop10* mutants
372 bolted around the 22nd day after germination whereas the inflorescence of wild-type bolted
373 gradually from the 23rd to the 26th day after germination (**Fig. 5D, Supplementary Table S8**).

374 Based on the mutant phenotype, we next examined the expression of three genes involved in
375 floral regulation, *SUPPRESSOR OF CONSTANS 1* (*SOC1*), *LEAFY* and *GA3OX-1*, in the
376 SAM of *proscop10* mutants and Col-0 at 7 and 11 days after germination (**Figure 6**). At 7
377 days, the two floral transition genes, *SOC1* and *LEAFY*, were not differentially expressed
378 between mutants and Col-0 whereas *GA3OX-1* was induced only in *proscop10-1*. At 11 days,
379 all three genes were up-regulated in the two *proscop10* mutant lines compared to Col-0.

380

381 **DISCUSSION**

382

383 **Two distinct hydroxylated SCOOP10 peptides are present in the leaf apoplasm**

384 The MS analysis of apoplastic fluid samples from rosette leaves has identified two hydroxylated
385 SCOOP10 peptides (18 and 20 aa-long) resulting from the processing of two distinct regions
386 of the PROSCOOP10 protein separated by 10 amino acids. This result confirms that
387 *PROSCOOP* genes are indeed encoding preproteins processed in short secreted peptides.
388 Furthermore, the presence of hydroxyproline residues confirms that SCOOPs are PTMPs
389 according to the Matsubayashi's classification (Matsubayashi, 2011). We thus characterized
390 two distinct SCOOP10 peptides showing the biological reality of the previous predicted
391 peptides SCOOP10#1 and SCOOP10#2. Note that the observed native peptides are a few
392 amino acids longer (at both their N- and C- termini) than previously predicted. As shown by
393 MS analyses, SCOOP10#2, corresponding to C-terminal end of the PROSCOOP10 precursor,
394 seems to be the major form in the leave apoplasm. The ability of a precursor protein to be
395 processed in different peptides has been described for a few genes belonging to CLE, CEP
396 and PIP families (Murphy *et al.*, 2012; Roberts *et al.*, 2013; Vie *et al.*, 2015). In our case, the
397 ability of *PROSCOOP10* to produce two distinct SCOOP10 peptides probably comes from the
398 local duplication of an exon. Indeed, contrary to the large majority of *PROSCOOP* genes which
399 have two coding exons (the first one encoding the signal peptide, and the second one
400 containing the conserved SCOOP motif), *PROSCOOP10* has a third exon containing a second
401 SCOOP motif (**Supplementary Fig. S1A**, Gully *et al.*, 2019). This feature is also shared by
402 *PROSCOOP6*, *7*, *11* and *15* which are also probably able to encode two SCOOP peptides
403 even if previous assays based on exogenous application of predicted synthetic peptides
404 showed that only the C-terminal ones have a biological activity (Hou *et al.*, 2021; Rhodes *et*
405 *al.*, 2021). The identification of these native SCOOP10 peptides suggests that their maturation
406 requires cleavage steps by endoproteases still unknown. The N-ter termini of both SCOOP10
407 peptides are located upstream the Y[KR]PN motif (**Figure 1**) similar to the cleavage site of IDA
408 precursors where P and Y residues in positions -2 and -4 relative to the cleaved bond are
409 important for cleavage site recognition by subtilases SBT4.12, SBT4.13 and SBT5.2
410 (Schardon *et al.*, 2016; Stintzi and Schaller, 2022). Because of its internal position in the
411 precursor, the release of SCOOP10#1 probably requires additional step involving actions of
412 another endoprotease and/or trimming by an exoprotease. Such complex maturation process
413 has already been described for the maturation of the CLE19 peptide through the activity of the
414 exoprotease Zn²⁺ carboxypeptidase SOL1 in the extracellular space (Casamitjana-Martinez *et*
415 *al.*, 2003; Tamaki *et al.*, 2013).

416 Regarding the post-translational modifications, the SCOOP10 amino acid motifs containing the
417 P hydroxylation sites were of three types: AP, TP, and GP. AP and TP are canonical motifs for
418 the hydroxylation of P residues described for the CEP1 peptide (Ohyama *et al.*, 2008) and

419 arabinogalactan proteins (Showalter *et al.*, 2010; Tan *et al.*, 2003). The GP motif was found to
420 be hydroxylated on the P residue in CLV3, CLE2 (Ohyama *et al.*, 2009), and in a few other
421 proteins (for a review see Canut *et al.*, 2016). As previously reported for other cell wall proteins,
422 the pattern of proline hydroxylation is variable (Duruflé *et al.*, 2017). It has been assumed that
423 this variability could contribute to the regulation of the biological activity or play a role in
424 recognition of the cleavage site(s) targeted by the endoproteases as Royek *et al.* (2022)
425 demonstrated with tyrosine sulfation. At this point, our data did not allow addressing the
426 question of the O-glycosylation of the hydroxyproline residues as reported for a few CLE
427 peptides (Shinohara and Matsubayashi, 2010, 2013; Araya *et al.*, 2014; Takahashi *et al.*, 2018)
428 and PSY1 (Amano *et al.*, 2007).

429 In our conditions, exogenous application of the synthetic SCOOP10#2 peptide, based on the
430 native forms with or without hydroxylated prolines (GDIFTGOSGSGHGGRTOAP or
431 GDIFTGPSGSGHGGRTPAP respectively), did not show any effect on seedling growth and
432 ROS production contrary to SCOOP12 and some other members of the family. However,
433 Rhodes *et al.* (2021) have shown that the predicted version of SCOOP10#2 peptide
434 (FTGPSGSGHGGR) induced a slight seedling growth inhibition and a low level of ROS at 1
435 μM in a MIK2 dependent manner. Hou *et al.* (2021), using another predicted sequence of the
436 SCOOP10#2 peptide (PNGDIFTGPSGSGHGGR, named SCOOP10#B in their publication),
437 have shown that it induced both a strong seedling growth inhibition and ROS production at the
438 same concentration and in a MIK2 dependent manner. These different results suggest that the
439 SCOOP10#2 actions regarding ROS production and seedling growth inhibition might be
440 sequence- and/or condition- dependent. The absence or the low effect of SCOOP10 peptides
441 on ROS production could explain why the constitutive high level of transcription of
442 *PROSCOOP10* in most of the aerial parts does not induce deleterious effects.

443 **SCOOP10 peptides tend to adopt a hairpin structure**

444 The mature sequence of SCOOP10 peptides being known, we addressed the question of its
445 molecular structure. NMR revealed that both synthetic SCOOP10#1 and SCOOP10#2 appear
446 to be mainly unstructured in solution. Molecular dynamics simulations also pointed to the
447 absence of a well-defined structure. Yet, the analysis of molecular dynamics trajectories
448 showed that the peptides transiently adopt a hairpin conformation, especially in the case of
449 SCOOP10#2. As often observed in ligand/receptor interactions, the active form of the protein
450 might be scarcely populated and become major only in the presence of its target (Pucheta-
451 Martinez *et al.*, 2016; Sekhar *et al.*, 2018). SCOOP10#2 transient structures would be
452 stabilized by two salt-bridges. The first one, between D2 and R16 side chains, favours the
453 formation of a turn (residues 8-9) exposing S8 and S10 while the second is between the N-

454 terminal amine and the C-terminal carboxylate. Despite the fact that the turn is not in the middle
455 of the structure, the two salt bridges can co-exist, because the three glycine residues in position
456 13-15 provide backbone flexibility. This stretch of glycines, located at the C-terminus of the S-
457 X-S motif, is a feature shared by a majority of the SCOOP peptides and allows us to think that
458 SCOOPs could adopt such a preferred conformation for the interaction with their receptor.
459 Interestingly, this hairpin structure exposes the two conserved serine residues that define the
460 SCOOP family and that were shown to be essential for the peptide function. Indeed, mutation
461 of one of the two residues is fatal for SCOOP12 perception (Gully *et al.*, 2019).

462

463 ***PROSCOOP10* delays the floral transition**

464 The mutation of *PROSCOOP10* showed an early flowering phenotype and a lower number of
465 leaves at bolting day compared to Col-0 plants. This observation indicates the involvement of
466 the *PROSCOOP10* gene in flowering-time control. Multiple factors alter the flowering time such
467 as the photoperiod, the vernalization, and the gibberellins (GAs). The pathways dependent on
468 these factors regulate a common set of key floral integrators (Parcy *et al.*, 2004; Moon *et al.*,
469 2005; Roux *et al.*, 2016; Li *et al.*, 2010). Among them, we tested the expression of the two major
470 genes *SOC1* and *LEAFY* (Simpson *et al.*, 2002; Parcy *et al.*, 2005), and of *GA3OX1-3*, involved
471 in the GA biosynthetic pathway and promoting flowering (Blázquez *et al.*, 1998). After 11 days,
472 when the floral transition occurred (Klepikova *et al.*, 2015), all three genes were up-regulated
473 in the two early flowering *proscoop10* mutants compared to Col-0. This suggests that
474 *PROSCOOP10* delays flowering time by repressing the expression of *SOC1*, *LEAFY* and
475 *GA3OX1-3*. Moreover, these results can be correlated with the *PROSCOOP10* expression
476 profile in the aerial parts of the plant. The mining of transcriptomic data available in
477 Genevestigator and CATdb (Zimmermann *et al.*, 2004; Gagnot *et al.*, 2008) revealed more
478 information about the transcriptional regulation of *PROSCOOP10*. Indeed, in 2007, Moon *et al.*
479 *al.* reported it as one of the only two genes significantly down regulated in the *pif1-5* mutant
480 while describing the involvement of *PIF1* in the optimization of the greening process through
481 the regulation of chlorophyll synthesis. Interestingly in 2018, Wu *et al.* also studied *PIF1* (called
482 *PIL5*) and identified an early flowering phenotype under long day growth condition for the *pil5-*
483 *1* mutant in which *SOC1* and *LEAFY* are also up-regulated. Moreover, Klepikova *et al.* (2015)
484 have monitored the transcriptome of the SAM to reveal a critical time point in Arabidopsis
485 flower initiation. In their analysis, *AT5G44580*, now identified as *PROSCOOP10* (Gully *et al.*,
486 2019), is highly transcribed in the SAM during the first 9 days after germination, then the
487 expression strongly decreased between 9 and 10 days, with a log₂ fold change of -4.54,
488 ranking 30 out of 968 down-regulated genes between these two stages. *PROSCOOP10*
489 expression remains at this low level at later stages of development. This expression profile is

490 intriguingly similar to that of *FLOWERING LOCUS (FLC)* another flowering regulator and
491 opposite to *LEAFY* whose expression increase during the transition to flowering (Klepikova *et*
492 *al.*, 2015). While the expression of *PROSCOOP10* in leaves seems rather constitutive, its
493 strikingly different expression profile in the SAM could fit with the early flowering phenotype of
494 the *proscoop10* mutants. Additionally, the transcriptomic profiles of *PROSCOOP10* in various
495 mutants or experimental conditions always showed a negative correlation between the level of
496 *PROSCOOP10* expression and the time span before flowering (**Table 1**). Indeed, studies have
497 shown that *PROSCOOP10* is repressed in plants showing an early flowering phenotype such
498 as in the *abi4vtc2* and *phyABCDE* mutants compared to their corresponding wild-type (Foyer
499 *et al.*, 2012; Hu *et al.*, 2013). Furthermore, in mutants such as *vtc2*, *tcp4* and *ga1-3* which
500 display a late flowering phenotype or no flowering at all, *PROSCOOP10* is induced (Blazquez
501 *et al.*, 1998; Pavet *et al.*, 2005; Kubota *et al.*, 2017). In the *arf6-2/arf8-3* mutant, inflorescence
502 stems elongate less than those of the wild-type and flowers arrest as infertile closed buds with
503 short petal. In this mutant, *PROSCOOP10* is also highly expressed in comparison with the
504 wild-type (Nagpal *et al.*, 2005). All these results suggest that *PROSCOOP10* interferes with
505 the floral transition process, upstream of *SOC1* and *LEAFY*, to delay flowering. Then,
506 *PROSCOOP10* could be another player of the meristem identity as a delayer of floral transition,
507 the actual signalling cascade remaining unknown. A putative role in the maintenance of the
508 vegetative state could also be suggested after the work published by Moon *et al.* in 2007, as
509 *PROSCOOP10* was one of the two genes co-regulated with *PIF1*, itself also reported as
510 optimizing the greening process. The fact that *PROSCOOP10* is rather constitutively
511 expressed in the green parts of the plant could support this hypothesis, as well as its huge
512 decrease of expression when the SAM acquires its floral identity (Klepikova *et al.*, 2015). The
513 involvement of *PROSCOOP10* and the respective functions of both *SCOOP10#1* and
514 *SCOOP10#2*, that we have identified, need further investigations to clarify their roles in these
515 mechanisms and identify their target and downstream signalling cascade. Altogether, these
516 data may link *PROSCOOP10* to the flowering-time control or the floral transition and illustrate
517 the functional complexity of the *PROSCOOP* family.

518

519 **Table 1: Expression profile of the *PROSCOOP10* genes extracted from public data**

Condition	Phenotype	<i>PROSCOOP10</i> transcription	Reference
10 th vs 9 th day, in SAM wild-type	wild-type	Down-regulated	Klepikova <i>et al.</i> , 2015
<i>pif1-5</i> vs wild-type	early flowering	Down-regulated	Moon <i>et al.</i> , 2007 ; Wu <i>et al.</i> , 2018
<i>abi4vtc2</i> vs wild-type	early flowering	Down-regulated	Foyer <i>et al.</i> , 2012
<i>phyABCDE</i> vs wild-type	early flowering	Down-regulated	Hu <i>et al.</i> , 2013
brassinolide treatment on wild-type	early flowering	Down-regulated	Li <i>et al.</i> , 2010
MeJA treatment on wild-type	early flowering	Down-regulated	Pak <i>et al.</i> , 2009
35S:: <i>ARAF1</i> vs wild-type	late flowering	Up-regulated	Chavez <i>et al.</i> , 2008
<i>vtc2</i> vs wild-type	late flowering	Up-regulated	Pavet <i>et al.</i> , 2005
<i>tcp4</i> vs wild-type	late flowering	Up-regulated	Kubota <i>et al.</i> , 2017
<i>ga1-3</i> vs wild-type	no flowering	Up-regulated	Blazquez <i>et al.</i> , 1998
<i>arf6-2/arf8-3</i> vs wild-type	immature flowers	Up-regulated	Nagpal <i>et al.</i> , 2005

520

521

522 SUPPLEMENTARY DATA

523 **Supplementary Table S1:** Primer sets for genotyping

524 **Supplementary Table S2:** ¹H and ¹³C NMR assignment of SCOOP10#2* peptide 1 mM in 50
525 mM phosphate buffer (10% D₂O), pH 6.6, 278K

526 **Supplementary Table S3:** ¹H and ¹³C NMR assignment of hydroxylated SCOOP10#2 peptide
527 0.5 mM in 50 mM phosphate buffer (10% D₂O), pH 6.6, 278 K

528 **Supplementary Table S4:** ¹H and ¹³C NMR assignment of SCOOP10#2* peptide 0.5 mM in
529 DMSO, 298K

530 **Supplementary Table S5:** ¹H and ¹³C NMR assignment of hydroxylated SCOOP10#2 peptide
531 0.5 mM in DMSO, 298K

532 **Supplementary Table S6:** ¹H and ¹³C NMR assignment of hydroxylated SCOOP10#1 peptide
533 0.5 mM in 50 mM phosphate buffer (10% D₂O), pH 6.6, 278K

534 **Supplementary Table S7:** ¹H and ¹³C NMR assignment of hydroxylated SCOOP10#1 peptide
535 0.5 mM in DMSO, 298K

536 **Supplementary Table S8:** Number of bolted inflorescences for Col-0 and *proscop10* mutants
537 per day after germination and per repetition

538

539 **Supplementary Figure S1: Genotyping of *proscoop10* mutant lines**

540 (A) Position of the T-DNA insertion in the *proscoop10-1* mutant. (B) On the left, check of the
541 T-DNA insertion by PCR on *PROSCOOP10* and comparison with PCR on *AtCOP1*, used as a
542 PCR positive control, in DNA of Col-0 and *proscoop10-1* mutant. On the right, check of
543 *PROSCOOP10* expression impairment in *proscoop10-1* mutant compared to Col-0 wild-type,
544 with *AtCOP1* used at RT-PCR positive control. (C) Position of the two RNA guides targeting
545 SCOOP10#1 and SCOOP10#2 regions in the *proscoop10-2* mutant. (D) Reference sequence
546 of PROSCOOP10. In red, the start and stop codons; in orange, the primer used for PCR
547 amplification and sequencing after cloning; highlighted in grey, the guides RNAg1 and RNAg2
548 and in bold, the PAM1 and PAM2. (E) Sequencing revealed two different alleles in the therefore
549 bi allelic *proscoop10-2* mutant. Alignments with the reference sequence shown for the first
550 mutated allelic version a deletion of 237 bp between the two guides, in addition to nucleotide
551 modifications in guides RNAg1 and RNAg2. This led to a deletion into SCOOP10#1 sequence
552 and a frameshift generating a stop codon and eliminating the SCOOP10#2 sequence. For the
553 second mutated allelic version, an important deletion of 448 bp has been generated between
554 the two guides, leading to a break in the two sequences corresponding to SCOOP10#1 and
555 SCOOP10#2 and a fusion of the remaining sequences. (F) Amino acid sequences of
556 PROSCOOP10 in *proscoop10-2* bi allelic mutant after CRISPR/Cas9 edition. For (A) and (C)
557 exons (CDS) and introns are represented by boxes and lines respectively. For (A), (C) and
558 (F) purple: SCOOP10#1 region; blue: SCOOP10#2 region; green: signal peptide; red star: stop
559 codon.

560 **Supplementary Figure S2: MS data related to SCOOP10#1 and SCOOP10#2 originating**
561 **from PROSCOOP10 identified in rosette leaves apoplastic fluids**

562 (A-H) For each peptide, a representative MS/MS spectrum is shown. The positions of the
563 hydroxyproline (O)/proline (P) residues are highlighted using the fragmentation data from the
564 C-terminus of each peptide (y ions). The information describing the experimental procedure is
565 available in Material and Methods.

566 **Supplementary Figure S3: NMR assignment and cis/trans isomerization of SCOOP10#2**
567 **in DMSO.** (A) NMR assignment of SCOOP10#2 reported on 1H,1H-TOCSY spectrum (HN/H α
568 region). (B) 1H,1H-NOESY cross-peaks between H α protons of G6 and H α or H α protons of
569 hydroxyproline in position 7 indicating the presence of both cis and trans conformation of the
570 G6-P7 peptide bond, respectively.

571 **Supplementary Figure S4: MD simulations of SCOOP10#2 mutants.** (A) DSSP secondary
572 structures calculated along the trajectories. (B) Contact maps of selected mutants.

573 **Supplementary Figure S5: Representative structures found in MD simulations for**
574 **SCOOP10#2 and its mutants.** For each structure the tube (left) and ribbon (right) renderings
575 are shown.

576 **Supplementary Figure S6: Structural behaviour of SCOOP10#1 in solution as monitored**
577 **by NMR.** Chemical shifts deviations from random coil values of H α protons and of the
578 difference between C α and C β carbons suggest the absence of a well definite structure for
579 SCOOP10#1. Deviations for glycine H α atoms were intentionally omitted.

580 **Supplementary Figure S7: Secondary structures and intramolecular interactions found**
581 **in MD simulations of SCOOP10#1.** (A) DSSP secondary structures calculated along
582 molecular dynamics (MD) simulation of hydroxylated SCOOP10#1 in solution. (B) Occurrence
583 of intramolecular polar atom contacts (H-bonds and salt bridges) in SCOOP10#1 calculated
584 along MD simulation trajectories. (C) Contact map.

585

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593 **AUTHOR CONTRIBUTIONS**

594 SA (project coordinator), J-PR and EJ designed and supervised the experiments. M-CG, EV
595 and MA-A performed the mutant production, phenotyping, peptide assays, and RT-qPCR
596 analysis. TB, MZ, EJ, HC and JC performed MS analysis of apoplastic fluids. CH-L, ND'A, FR-
597 M and ER analysed peptide structure through RMN and molecular dynamics. M-CG, ND'A,
598 ER, J-PR, EJ and SA wrote the manuscript and all authors read and approved the final version.

599 **CONFLICT OF INTEREST**

600 The authors declare no competing interest.

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607 **DATA AVAILABILITY**

608 All data supporting the findings of this study are available within the paper and within its
609 supplementary materials published online. Biological materials are available from the
610 corresponding authors upon request.

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823

824 **FIGURE LEGEND**

825 **Figure 1: Identification of two SCOOP10 peptides by MS.**

826 (A) Sequence of the prepropeptide PROSCOOP10. The predicted signal peptide is in green, the mature
827 SCOOP10#1 in purple and the mature SCOOP10#2 in blue. The tryptic cut sites (arginine (R) and lysine
828 (K)) are indicated with yellow discs. The proline residues which were found to be hydroxylated are in
829 black and underlined (P). The conserved serine residues are in bold. (B) Description of the different
830 peptides covering the SCOOP10#1 and SCOOP10#2 amino acid sequences. The positions of P
831 hydroxylation are indicated with O which stands for hydroxyproline. Stars indicate that the peptide has
832 been identified after tryptic digestion. The frequency of observations of each peptide is indicated as well
833 as the percentage of occurrences. (C) A focus on SCOOP10#2 to show the number of observations and
834 the frequency of hydroxylation events at each P position.

835 **Figure 2: Structural behaviour of SCOOP10#2 and SCOOP10#2* in solution**

836 (A) ^1H , ^{13}C -HSQC spectrum assignment of non-hydroxylated SCOOP10#2* (blue) and hydroxylated
837 SCOOP10#2 (red) 0.5 mM in 50 mM phosphate buffer at pH 6.6 and 278 K. (B) Chemical shifts
838 deviations from random coil values of $\text{H}\alpha$ protons and of the difference between $\text{C}\alpha$ and $\text{C}\beta$ carbons
839 suggest the absence of a well definite structure for SCOOP10#2. Deviations for glycine $\text{H}\alpha$ atoms were
840 intentionally omitted.

841 **Figure 3: Secondary structures and intramolecular interactions found in MD simulations**
842 **of SCOOP10#2.**

843 (A) DSSP secondary structures calculated along molecular dynamics (MD) simulation of hydroxylated
844 SCOOP10#2 in solution; (B) Occurrence of intramolecular polar atom contacts (H-bonds and salt
845 bridges) in SCOOP10#2 calculated along MD simulation trajectories; (C, D) Schematic representations
846 of SCOOP10#2 shown as a 'tube' coloured from blue (N-terminus) to red (C-terminus). Side-chains are
847 shown as sticks with the following color code: positively charged (blue) and non-polar (light gray). The
848 structures were created with PyMol (DeLano *et al.*, 2002). Key intramolecular interactions are indicated
849 by two short parallel dashes.

850 **Figure 4: Effect of SCOOP10 peptides on seedling growth and on ROS production**

851 (A) Seedling growth inhibition evaluation by fresh mass measuring after 1 μM elicitor or control
852 treatment. (B) H_2O_2 production after 1 μM elicitor treatment or control treatment, measured with a
853 luminol-based assay using leaf discs from 4-week-old plants of the indicated genotypes. Data
854 represented are means of three independent replicates of over times RLU (relative luminescence units)
855 ($n=3$, $\pm\text{SEM}$). For (A) and (B), SCOOP10#1 and SCOOP10#2 correspond to the proline hydroxylated
856 peptides and prolines are not hydroxylated for the SCOOP10#2*peptide.

857 **Figure 5: *proscop10* early flowering phenotype.**

858 (A) Bolted inflorescence of the *proscop10* mutant lines, the 22nd day after germination, indicated by the
859 white arrows. (B) Delay of stem development between mutants and WT due to the early flowering of the
860 *proscop10* lines, at the 27th day after germination. Scale bar = 1cm for (A) and (B). (C) Average of day
861 to bolting for each genotype. (D) Kinetics represent the number of bolted inflorescences in %, starting
862 from day 20 to day 30 after germination. ($n=25$). (E) Average of the number of rosette leaves at the
863 flowering time for each genotype. For (C) and (E) ANOVA and Tukey test allowed to define significantly
864 two different groups labelled a and b.

865 **Figure 6: Impact of *PROSCOOP10* mutation on transcription of genes involved in floral**
866 **regulation**

867 Relative expression of *SOC1*, *LEAFY* and *GA3OX-1* genes was measured by RT-qPCR after 7 days (in
868 grey) and 11 days (in black) in the two *proscop10* mutant lines compared to Col-0 at 7 days. Values
869 represent mean ratios \pm SEM of three independent biological replicates. Asterisks denote statistical
870 differences of gene expression levels between *proscop10* mutants and Col-0 : * $P < 0.05$, ** $P < 0.01$
871 (ratio paired t-test).

872 **FIGURES**

873

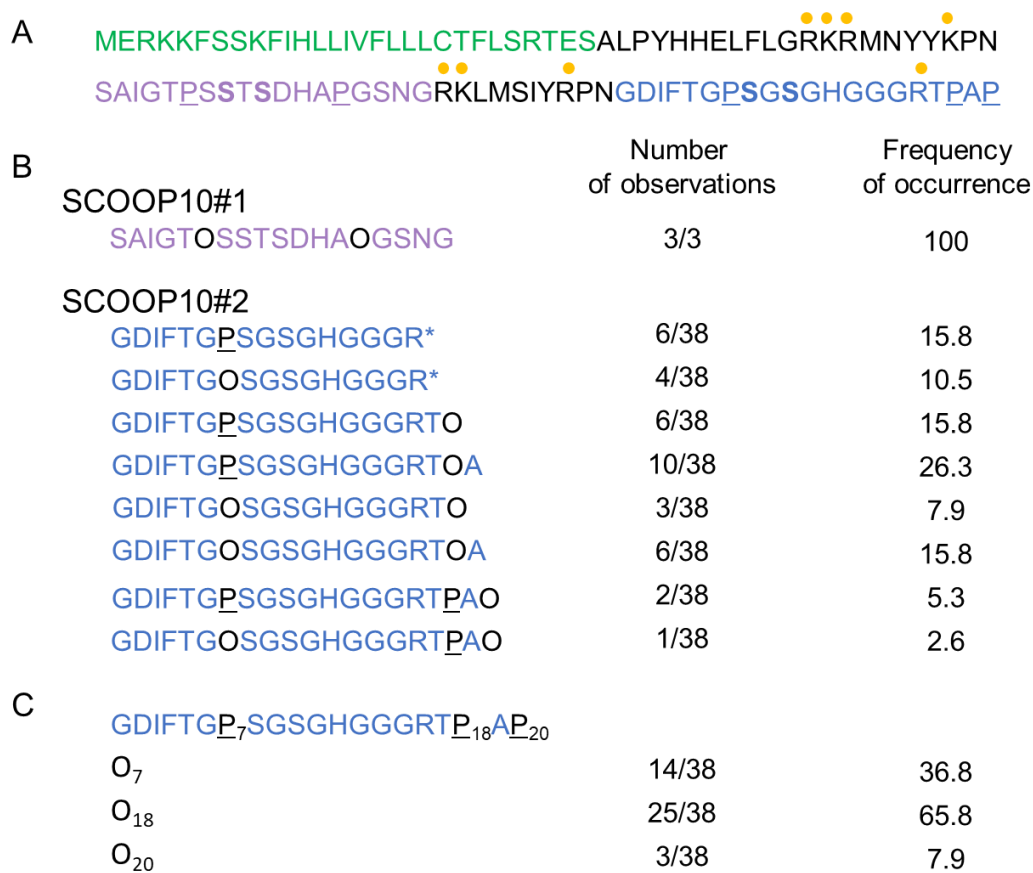


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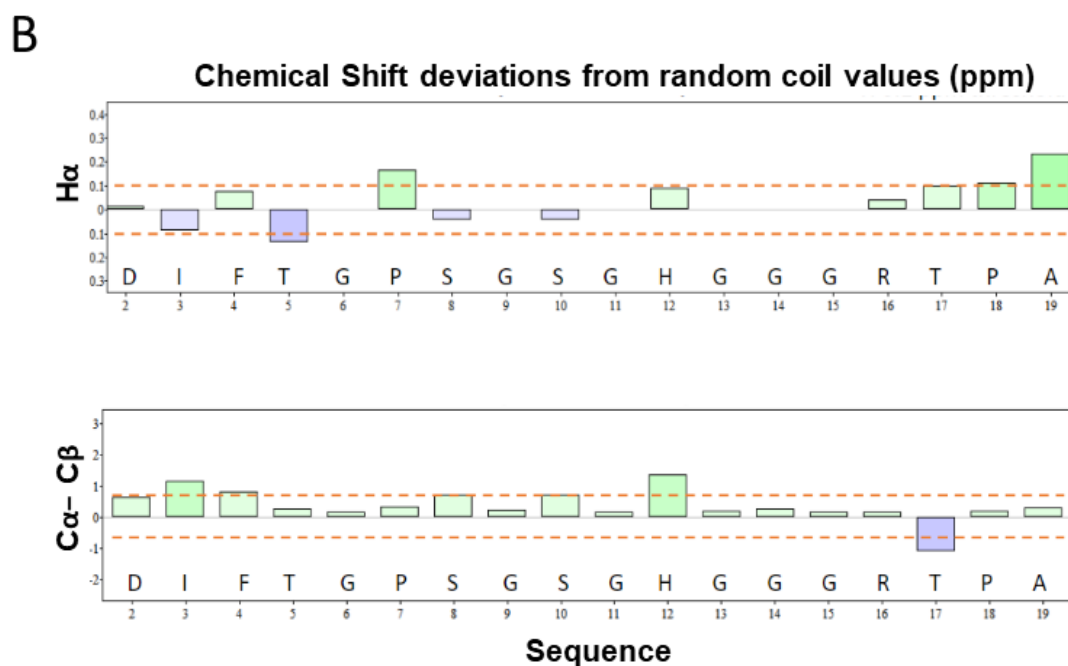
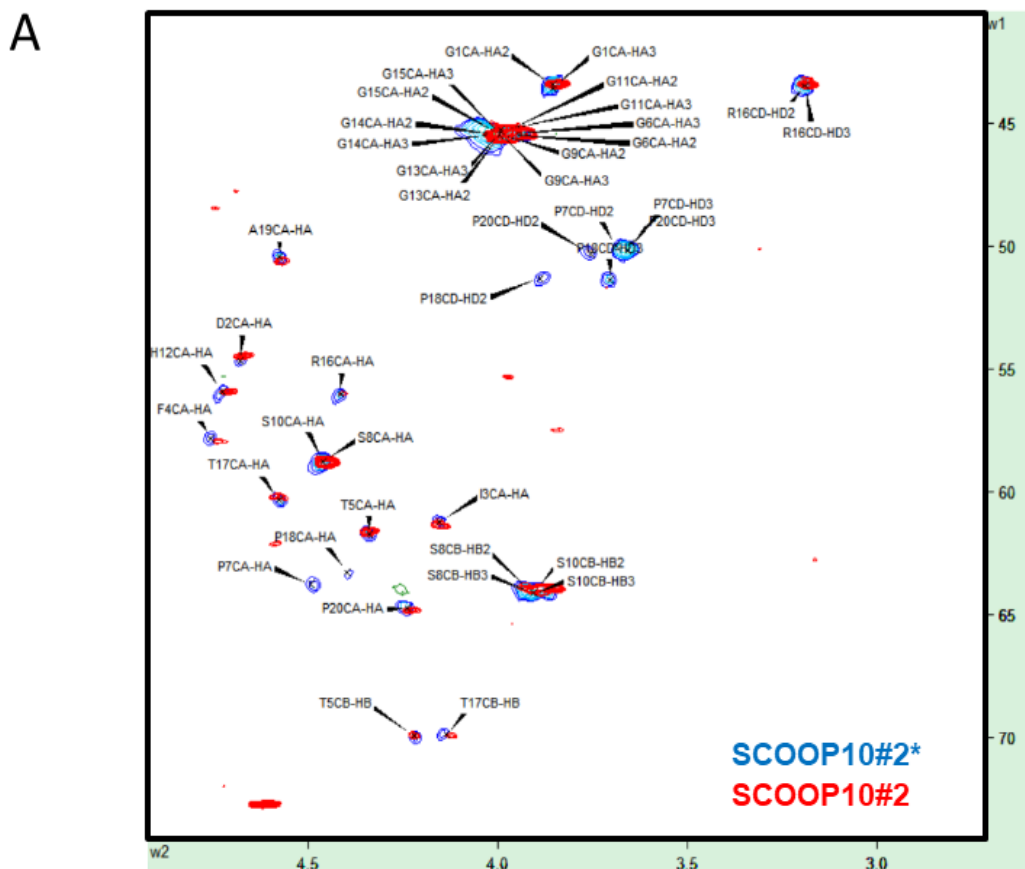


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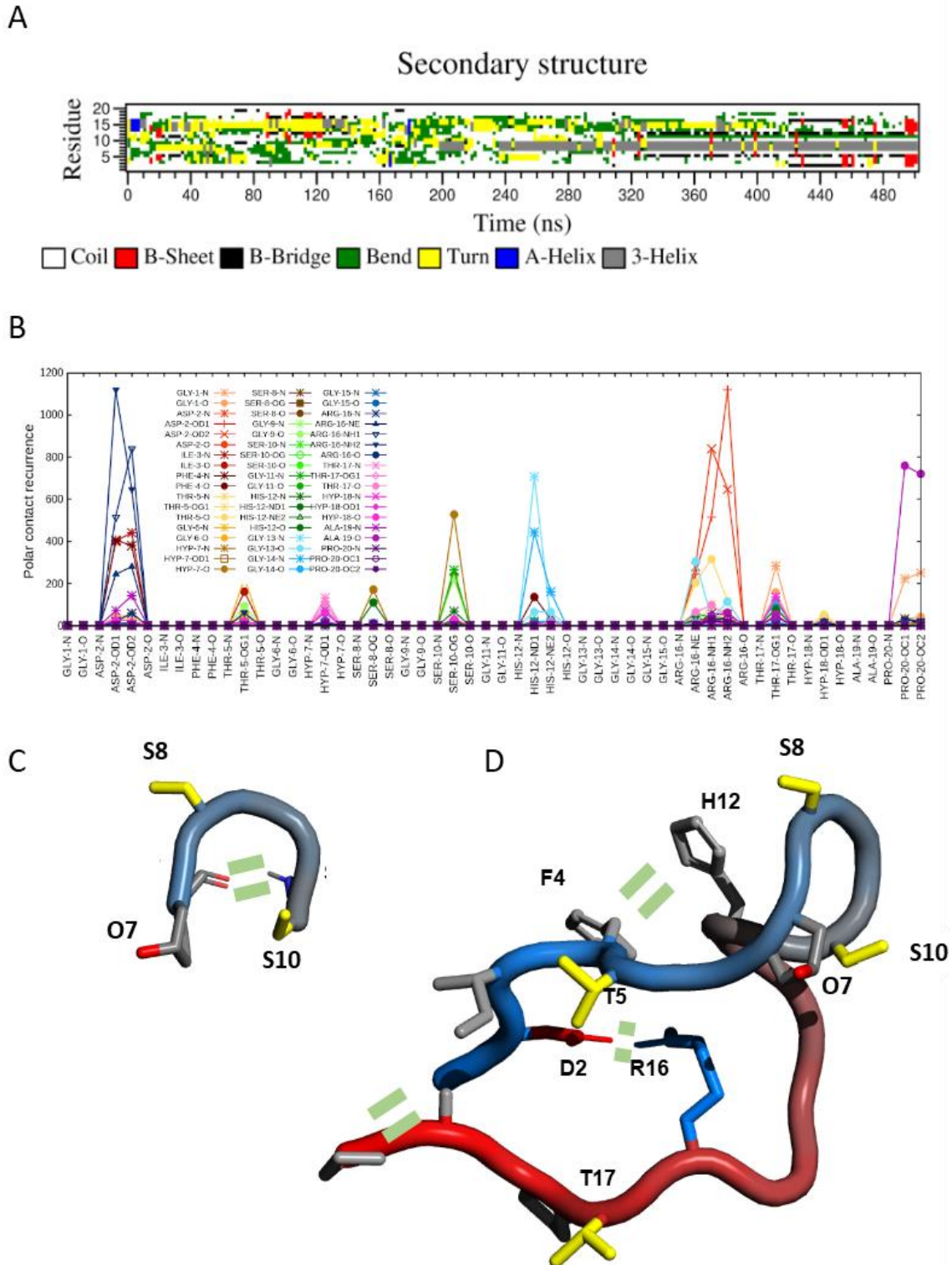


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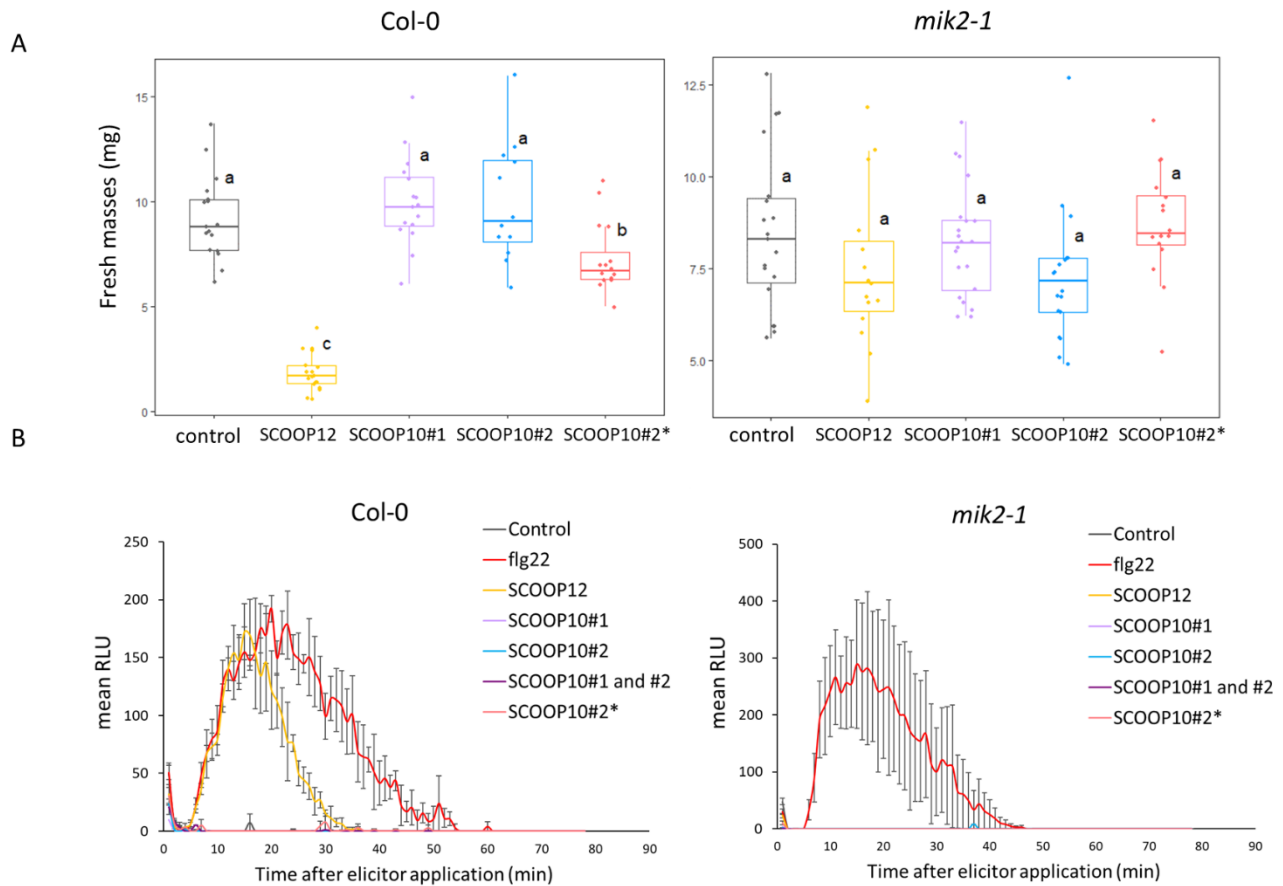


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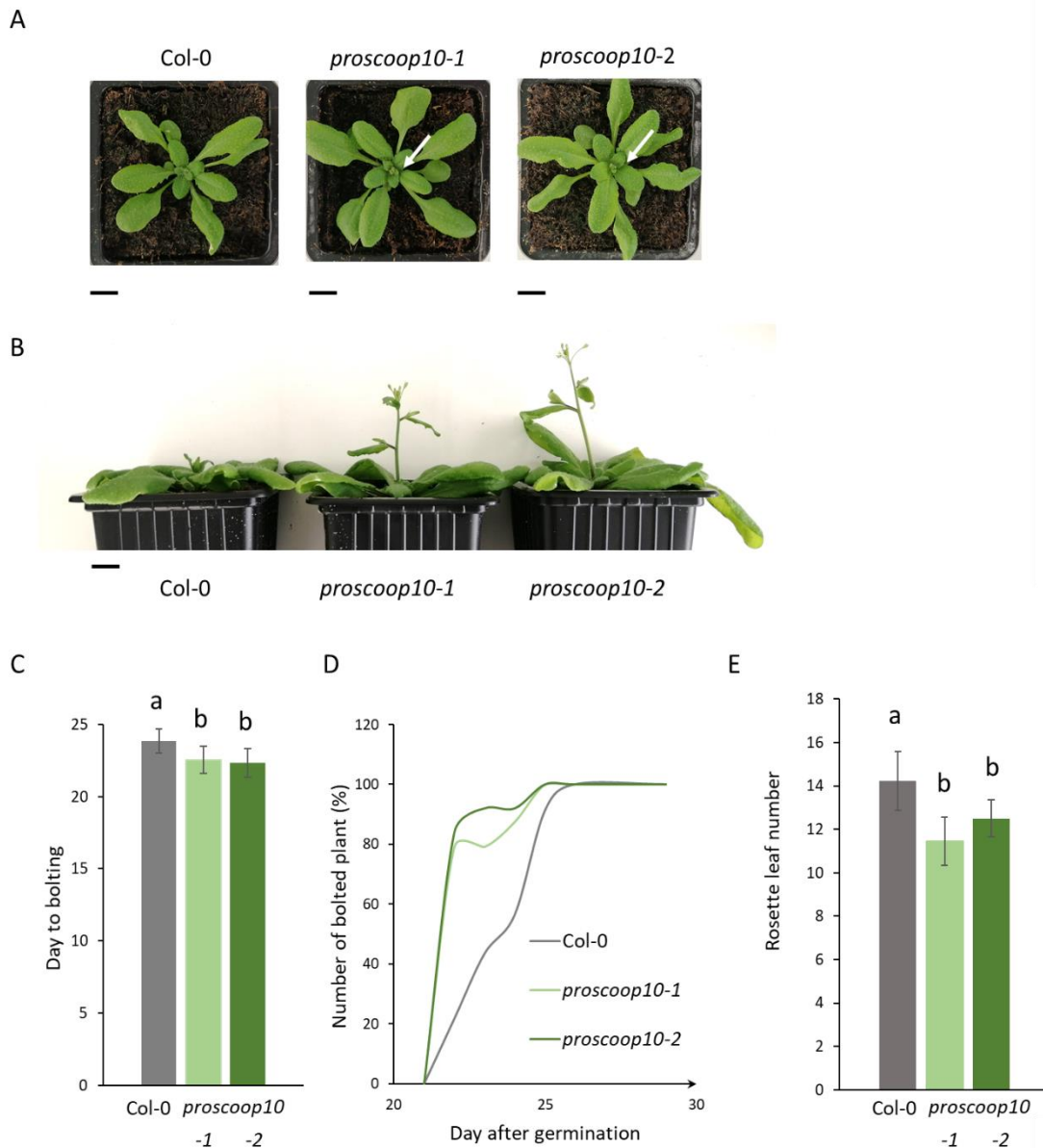


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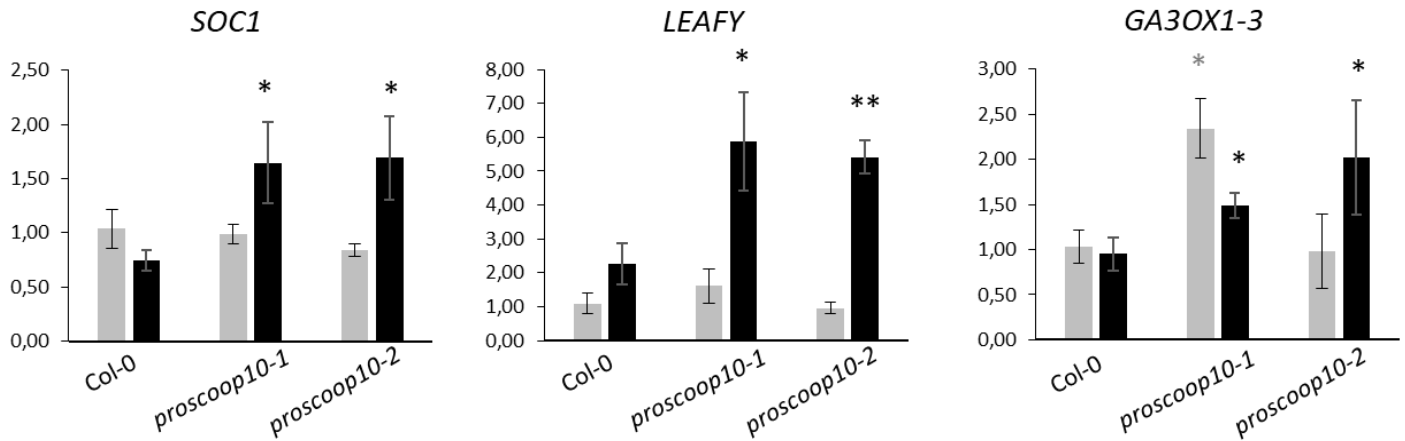


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