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## Rose FT homologous gene overexpression affects flowering and vegetative development behavior in two different rose genotypes

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1

2 **Rose *FT* homologous gene overexpression affects flowering and vegetative**  
3 **development behavior in two different rose genotypes**

4

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7

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9 France

10 **Key message** The overexpression of *RoFT* gene in two rose varieties induces pleotropic  
11 effects and up- or down-regulates several target genes.

12 **Keywords** *Rosa hybrida*, *FLOWERING LOCUS T* gene, genetic transformation

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## 15 **Abstract**

16 To address the major challenge of controlling flowering in roses, we studied the ectopic  
17 expression of a *FLOWERING LOCUS T* (FT) homolog, which plays an important role in  
18 flowering time regulation in plants. We detected seven rose genes of the phosphatidyl  
19 ethanolamine-binding protein (PEBP) family and selected one of them (*RoFT*) based on its  
20 co-localization with flowering and architecture QTLs to study its overexpression in two  
21 rose genotypes. Embryogenic lines were obtained and genetic transformation was  
22 performed on the rose genotypes. Regenerated plantlets were maintained in a greenhouse  
23 until flowering and then floral and architectural traits were scored. We observed  
24 substantial variation in phenotypic expression between genotypes and between events,  
25 especially in the flowering date and number of floral organs. The expression of 14 genes  
26 putatively involved in floral initiation (mainly *FT* targets) and floral development (from the  
27 class ABC model) was studied to gain insight into these variations. We noted that target  
28 genes are up- or down-regulation and can **partially** explain the observed phenotype. Our  
29 results revealed that a gene ectopically overexpressed in different genetic backgrounds  
30 could have diverse effects and that the overexpression *RoFT* gene can have pleiotropic  
31 effects.

32

## 33 **Introduction**

34 Flowering is a major trait of interest in the ornamental plant sector. In creating new  
35 cultivars, rose breeders aim to enhance this trait by i) controlling the flowering date  
36 (earliness or lateness), ii) extending the flowering period, and iii) selecting roses able to  
37 bloom continuously or under different environmental conditions. It is therefore essential  
38 to gain knowledge on the factors that influence the transition from the vegetative phase  
39 to the floral phase so as to be able to achieve this goal.

40 The current floral transition model is well known in the annual monocarpic species  
41 *Arabidopsis thaliana*, while reportedly involving a complex genetic network of about 300  
42 genes (Bouche et al. 2016; Quiroz et al. 2021). Flowering is thus controlled by endogenous  
43 and exogenous cues and the *FLOWERING LOCUS T* (*FT*) gene is considered to incorporate

44 inputs from several pathways, ultimately resulting in floral transition (for review Kinoshita  
45 and Richter 2020). Florigen-encoding *FT* genes have been extensively identified and their  
46 functions are known to be conserved among short day (SD), long day (LD) and day neutral  
47 (DN) plants (Pin and Nilsson 2012). *FT* belongs to the phosphatidylethanolamine-binding  
48 protein (PEBP) family. Members of this family control processes as diverse as seed  
49 development, growth cessation and seasonal growth control in trees, bud outgrowth and  
50 bolting, bulb formation, tuberization and flowering regulation (Bohlenius et al. 2006;  
51 Miskolczi et al. 2019; Navarro et al. 2011; Xi et al. 2010). In *A. thaliana*, *FT* and its paralog  
52 TWIN SISTER OF *FT* (*TSF*) promote flowering whereas other members of the PEBP family,  
53 especially TERMINAL FLOWER1 (*TFL1*), repress flowering in the vegetative and  
54 inflorescence meristem. Under inductive (e.g. LD) photoperiod conditions, *FT* is expressed  
55 in leaf phloem companion cells (PCCs) and the *FT* protein moved via the phloem to the  
56 shoot apical meristem (SAM) has been demonstrated to be the florigen molecule (for a  
57 review, see Putterill and Varkonyi-Gasic 2016; Srikanth and Schmid 2011). In the shoot  
58 apical meristem, the *FT* protein interacts with the transcription factor, *FD* and 14-3-3  
59 proteins which promote transition of the vegetative meristem into a reproductive  
60 inflorescence meristem (Abe et al. 2005; Taoka et al. 2011; Wigge et al. 2005) by  
61 increasing expression of the *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1*  
62 (*SOC1*) and the floral meristem identity genes (*APETALA 1 (API)*, *FRUITFULL (FUL)* and  
63 *LEAFY (LFY)*) and induce flowering (Collani et al. 2019; Jaeger and Wigge 2007; Wigge et  
64 al. 2005). Several studies in *A. thaliana* demonstrated that the MADS box transcription  
65 factor FLOWERING LOCUS C (*FLC*) is another major flowering regulator via the  
66 vernalization pathway which inhibits the transcription of floral promoters, including *FT*  
67 and *SOC1* (Helliwell et al. 2006; Searle et al. 2006).

68 In the ornamental plant sector, roses are classified amongst perennial shrubs. The  
69 *Rosa* genus includes more than 200 species and 30,000 cultivars which are distributed  
70 worldwide (Wissemann and Ritz 2007). The floral transition model is hard to transpose in  
71 rose because: i) rose is a perennial polycarpic species, contrary to *A. thaliana*, and ii) a  
72 major gene involved in floral transition (*FLOWERING LOCUS C, FLC*) has not been  
73 identified in rose (Raymond et al. 2018; Remay et al. 2009). Furthermore, modern rose

74 genotypes are mainly continuously flowering. These continuous-flowering roses feature  
75 determinate flowering and are considered as being day-neutral (DN).

76 Many publications have highlighted the impact of the overexpression or **extinction**  
77 **suppression** of homologous *FT* genes *in planta*. In perennial plants, *FT* gene  
78 overexpression induces early flowering in *Eucalyptus* (Klocko et al. 2016), poplar  
79 (Bohlenius et al. 2006) and apple (Tanaka et al. 2014). Other modifications may also be  
80 observed such as suppression of the short-day-induced growth cessation trait and bud set  
81 in *Populus* (Hsu et al. 2006; Wenzel et al. 2013) and variation in the number of floral  
82 organs in apple (Tanaka et al. 2014). In ornamental plants, constitutive expression of the  
83 *FT* gene from blueberry induces earlier flowering in petunia (Lin et al. 2019). Ectopic  
84 expression of the *FT* homologue from *Prunus mume* reduces the juvenile phase and early  
85 flowering in the non-recurrent rose cultivar *Rosa rugosa* 'Bao White'. Note that the early  
86 flowering observed in *Rosa rugosa* alters the multiplication and rooting capacity of some  
87 transformation events (Xing et al. 2014). Nakatsuka et al. (2009) used the *FT* gene from  
88 *Arabidopsis* to transform an ornamental gentian, which gave rise to an early flowering  
89 phenotype with normal flower development. In *Artemisia*, *FT* RNAi causes a delay in  
90 flowering time and an increase in artemisine content (Lv et al. 2018) while, in *Populus*,  
91 *FT* interference generates a setting that is more sensitive day length shortening and leads  
92 to earlier bud set (Bohlenius et al. 2006).

93 In rose, two *FT* and *TFL1* homologues, i.e. respectively *RoFT* and *RoKSN*, were  
94 **previously** characterized. *RoFT* is induced during floral transition, and has been proposed  
95 to lead to transcript accumulation of the *LFY* and *API* homologues (Randoux et al. 2014;  
96 Remay et al. 2009). *RoFT* co-localises with a QTL for the flowering date on linkage group  
97 3 (previously denominated 4), thereby showing that variability at the *RoFT* locus could be  
98 responsible for early or late flowering, associated with transcriptional regulation of *RoFT*  
99 (Iwata et al. 2011; Otagaki et al. 2015; Roman et al. 2015). Moreover, *RoFT* gene  
100 overexpression in *Arabidopsis* led to early flowering (Otagaki et al. 2015; Randoux et al.  
101 2014). The *TFL1* homologue, i.e. *RoKSN* is controlling the mode of flowering. Once-  
102 flowering roses have a functional allele leading to inhibition of blooming after to first  
103 flowering in spring until next spring (Iwata et al. 2011; Randoux et al. 2012). Different

104 alleles were detected leading to different modes of flowering. Two knock-out mutants are  
 105 responsible of continuous-flowering: one is due to the insertion a large *copia* transposable  
 106 element (*RoKSN<sup>copia</sup>* allele (Iwata et al. 2011)) and the second is a complete deletion of  
 107 the gene (*RoKSN<sup>null</sup>* allele, Hibrand Saint-Oyant et al. 2018). These two alleles are  
 108 responsible of the complete absence of *RoKSN* transcript leading to roses that flower  
 109 continuously. Intermediate phenotype (recurrent blooming roses that can rebloom late in  
 110 the season, but without a continuous flowering behaviour) were characterized with other  
 111 *RoKSN* alleles: the *copia* in the *RoKSN<sup>copia</sup>* allele can recombine and generate a new  
 112 allele with only a LTR, Long Terminal Repeat (*RoKSN<sup>LTR</sup>*, Iwata et al. 2011). A conversion  
 113 of G into a A at position 181 of the CDS is also responsible of recurrent blooming  
 114 (*RoKSN<sup>A181</sup>* (Soufflet-Freslon et al. 2021)). *RoKSN* overexpression in rose totally blocked  
 115 flowering in a continuous flowering genotype. This non-flowering phenotype might have  
 116 been due to complete repression of the transcript accumulation of *RoFT* and/or to  
 117 competition between *RoFT* and *RoKSN* for interaction with *RoFD* (Randoux et al. 2014).

118 We used the rose genome sequence to study the PEBP family in rose and to  
 119 functionally validate a *RoFT* homologue by overexpressing it in two rose genotypes. In  
 120 transgenic plants, we studied the following traits: flowering time, architecture and floral  
 121 organs. We also studied the effect of this overexpression on transcript accumulation of  
 122 genes involved in floral initiation and development.

123

## 124 **Materials and Methods**

### 125 **Plant material**

126 Two continuous-flowering genotypes were used in the present study, i.e. *Rosa* DELdog  
 127 ‘Pimprenelle®’ [PIMP], and *Rosa* DELtrimen ‘Guy Savoy®’ [GS], which were obtained  
 128 from the *Société Nouvelle des pépinières Georges Delbard* (Malicorne, France). These  
 129 genotypes are tetraploid cultivated garden roses (2X=4N=28). The *in vitro* conditions for  
 130 introduction, multiplication and maintenance are as described in Hamama et al. (2019).

131 The plants were introduced in *in vitro* culture from meristems. Explants were cultured  
 132 and sub-cultured on Murashige et Skoog (MS) (Murashige and Skoog 1962) basal medium  
 133 complemented with 0.05 g.L<sup>-1</sup> Fe-EDDHA, 0.1 mg.L<sup>-1</sup> GA<sub>3</sub> and 0.5 mg.L<sup>-1</sup> BAP (6-

134 benzylaminopurine), sucrose 30 g.L<sup>-1</sup> and solidified with 3 g.L<sup>-1</sup> Phytigel™ (Sigma, Ref:  
135 P8169) (shoot elongation medium, SEM). The pH medium pH was adjusted to 5.7 before  
136 sterilization (113°C, 20 min). The cultures were conducted under a 16 h photoperiod with  
137 a photosynthetic flux (PAR) of 56.4 μmol m<sup>-2</sup> s<sup>-1</sup> (generated by a combination of two  
138 Sylvania Luxline F58W/840 fluorescent lamps and one Osram Biolux L 58W/72-965  
139 fluorescent lamp). The temperature regime was 23 ± 0.5 °C during the 16 h light period  
140 and 19 ± 0.5 °C during the 8 h dark period.

### 143 **RoFT isolation and cloning**

#### 144 *Phylogenetic tree construction, gene structure and protein motif analysis*

145 PEBP homologues were isolated from the reference genome (Hibrand Saint-Oyant  
146 et al. 2018) by BLAST (Altschul et al. 1990; Camacho et al. 2009) using previous a  
147 characterized rose *FT* gene (Remay et al. 2009). A phylogenetic tree was constructed  
148 from PEBP-predicted PEBP protein sequences using the Geneious Prime (2022.2.1)  
149 software platform. Multi-alignment was done using the MUSCLE 3.8.425 app (Edgar  
150 2004). After manual multi-alignment correction, the tree was built using the Geneious  
151 Tree Builder by the Neighbor-joining (NJ) method with 1,000 bootstrap replicates. The  
152 predicted protein sequences were obtained from published genome available on the GDR  
153 website (Jung et al. 2019), i.e. the haploid *Rosa* genome sequence (Hibrand Saint-Oyant  
154 et al. 2018), the haploid *Malus* GDDH13 reference genome sequence (Daccord et al. 2017)  
155 and the Peach reference genome sequence (Verde et al. 2017). The micro-synteny analysis  
156 was done using synteny viewer tools developed on the GDR website  
157 (<https://www.rosaceae.org/synview/search> (Jung et al. 2019)). The analysis is done using  
158 MCScanX (Wang et al. 2012).

#### 160 *Cloning into the pK7GWS vector and Agrobacterium transformation*

161 The *RoFT* gene was amplified from leaf cDNA from a *Rosa wichurana* hybrid, as described  
162 in Randoux et al. (2014) and cloned using the Gateway® system (Invitrogen) with the  
163 TOPO isomerase mix into the pENTR™ / D-TOPO™ entry vector. For plant transformation,



164 *RoFT* cDNA (534 bp) was introduced by LR Clonase recombination into the pK7WG2D  
165 vector [provided by the University of Gent \(http://gateway.psb.ugent.be/\)](http://gateway.psb.ugent.be/).

166 The pK7WG2D/*RoFT* binary vector was introduced by electroporation into the  
167 *Agrobacterium tumefaciens* EHA105 strain (Hood et al. 1993) containing the pBBR  
168 plasmid.

169

## 170 **Rose genetic transformation**

171 *Somatic embryo formation, multiplication, germination and plantlet formation*

172 Somatic embryo lines of the two genotypes were established using leaflets from 6  
173 week old vitroplants maintained on MS elongation medium as described in Hamama et al.  
174 (2019). Young fully developed leaves were cut into 0.5 to 0.75 cm disc diameters. For  
175 callus induction, leaf disks were wounded and incubated abaxial side down in Petri dishes  
176 containing callus induction medium (CIM), i.e. MS basal medium containing 2 mg/L 2,4D  
177 ([Duchefa Biochemie, Ref: D0911.0256](#)), 30 g/L sucrose ([Duchefa Biochemie, ref: S0809.5000](#)) and 3 g/L Phytigel™. All ingredients were added to the medium before  
178 autoclaving (at 113°C for 20 min). The pH was adjusted to 5.8. Cultures were incubated  
179 in the dark at 22°C for two 1-month subcultures. The obtained calli were isolated and  
180 maintained on MS medium containing 1 mg /L 2,4D for two subcultures in the same  
181 conditions as above.

182 Compact yellow embryogenic calli were then transferred and maintained on embryo  
183 induction medium (EIM) (Vergne et al. 2010). EIM consisted of MS medium containing  
184 zeatin ([Duchefa Biochemie, ref: 015494.04](#)) 4 mg/L. Embryogenic lines were maintained  
185 by secondary somatic embryogenesis formation. Embryogenic callus lines were  
186 subcultured for 6 weeks under low light intensity 20  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

187 ~~Secondary somatic embryos at cotyledonary stage were used as targeted tissues for~~  
188 ~~*Agrobacterium*-mediated transformation.~~

189 ~~Secondary somatic embryos were used as targeted tissues for *Agrobacterium*-mediated~~  
190 ~~transformation.~~

191 ~~Shoot regeneration was induced by subculturing well-developed secondary somatic~~  
192 ~~embryos on shoot multiplication medium (SMM) consisting of Murashige and Skoog basal~~  
193

194 medium containing 30 g/L of sucrose and 3 mg/L BAP. The explants were then subcultured  
195 every 3 weeks under 70  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light intensity at 23°C and for 16 h and in the dark  
196 at 19°C for 8 h. Young regenerated shoots were transferred onto shoot elongation medium  
197 (SEM) consisting of MS medium supplemented with BAP 0.5 mg/L and GA3 0.1 mg/L  
198 (Hamama et al. 2019). For rooting, plantlets were transferred on half dilution MS medium  
199 free of growth regulator. All ingredients were added to the medium before autoclaving (at  
200 113°C for 20 min). The pH was adjusted to 5.8.

201 After rooting, the plantlets were transferred on motte fertiss® then maintained for one  
202 month in an acclimation chamber (16h/8h light at 22°C) before subculturing into substrate  
203 adapted to rose (Formule Potees Sub Big-Bag, ref 391796) in a S2 greenhouse in the  
204 general following conditions: minimum air temperature maintained at 18 °C, with an  
205 aeration at 20 °C; relative humidity maintained at 70% and no complementary lighting.  
206 To homogenise them, the plants are kept in the greenhouse for between 2 and 6 months,  
207 then pruned and placed in a cold room (6°C±2°C) for 6 weeks before being returned to  
208 the greenhouse until they flower at which stage, the samples are taken.

### 210 *Agrobacterium transformation*

211 For rose, the transformation process was adapted from Dohm et al. (2001) and Vergne et  
212 al. (2010) for the GS genotype. The transformation process for the PIMP genotype is  
213 described below.

214 One 2-day-old colony of the EHA105 *Agrobacterium tumefaciens* strain containing the  
215 plasmid of interest was spread on LB solid medium supplemented with spectinomycin  
216 (Duchefa, ref: S0188.0005) (50 mg/L), gentamycin

217 (100 mg/L) and rifampicin (Duchefa, ref: R0146-0005) (50 mg/L) for 2 days. A suspension  
218 of *Agrobacterium* in MinA medium (Svab and Hajdukiewicz 1975), pH 5.6, containing  
219 acetosyringone (Aldrich, ref: D134406-1G)

220 (100  $\mu\text{M}$ ) ( $\text{OD}_{600} = 1$ ) was cultivated for 2 h at 28°C with 150 rpm agitation to induce *Vir*  
221 genes. Somatic embryo clusters at the cotyledonary stage were infected by the  
222 *Agrobacterium* suspension ( $\text{OD}_{600}$ =0.1-0.5) and transferred onto EIM for a 1 day co-  
223 cultivation step in the dark (21±2°C). Cultures were then transferred on EIM selection

224 medium containing kanamycine ([Duchefa, ref: K0126.0025](#)) (100 mg/L) and cefotaxime  
225 ([Duchefa, ref: C0111.0025](#)) (500 mg/L) to eliminate bacteria. The embryogenic calli were  
226 subcultured on fresh EIM selection medium every 3 weeks until the formation of vigorous  
227 kanamycine-resistant secondary somatic embryos. Germination and shoot regeneration  
228 were achieved on MS medium containing 40 g/L maltose as carbohydrate source with a  
229 modified MS nitrogen composition (NH<sub>4</sub>NO<sub>3</sub> 9.4 mM; KNO<sub>3</sub> 20.6 mM). ~~Each well-formed~~  
230 ~~plantlet was identified and transferred on selection elongation medium before rooting, as~~  
231 ~~described in Hamama et al. (Hamama et al. 2019). Shoot regeneration was induced by~~  
232 ~~subculturing well-developed secondary somatic embryos on shoot multiplication medium~~  
233 ~~(SMM) consisting of MS basal medium containing 30 g/L of sucrose and 3 mg/L BAP (6-~~  
234 ~~benzylaminopurine, Duchefa Biochemie, ref: B0904.0001). The explants were then~~  
235 ~~subcultured every 3 weeks under 70 μmol m<sup>-2</sup> s<sup>-1</sup> light intensity at 23°C and for 16 h and~~  
236 ~~in the dark at 19°C for 8 h. Young regenerated shoots were transferred onto shoot~~  
237 ~~elongation medium (SEM) consisting of MS medium supplemented with BAP 0.5 mg/L~~  
238 ~~and GA3 (SIGMA, ref: 201-001-0) 0.1 mg/L (Hamama et al. 2019). For rooting, plantlets~~  
239 ~~were transferred on half dilution MS medium free of growth regulator. All ingredients~~  
240 ~~were added to the medium before autoclaving (at 113°C for 20 min). The pH was adjusted~~  
241 ~~to 5.8.~~

242 ~~After rooting, the plantlets were transferred on motte fertiss® then maintained for one~~  
243 ~~month in an acclimation chamber (16h/8h light at 22°C) before subculturing into substrate~~  
244 ~~adapted to rose (Formule Potees Sub Big-Bag, ref 391796) in a S2 greenhouse in the~~  
245 ~~general following conditions: minimum air temperature maintained at 18 °C, with an~~  
246 ~~aeration at 20 °C; relative humidity maintained at 70% and no complementary lighting.~~  
247 ~~To homogenise them, the plants are kept in the greenhouse for between 2 and 6 months,~~  
248 ~~then pruned and placed in a cold room (6°C±2°C) for 6 weeks before being returned to~~  
249 ~~the greenhouse until they flower at which stage, the samples are taken.~~

250 ~~Greenhouse acclimatisation was performed.~~

251 Hundred embryogenic clusters for the GS genotype and four replicates (11, 13, 18, 16  
252 embryogenic clusters) for PIMP were inoculated with *Agrobacterium* harbouring the  
253 pK7WG2D/RoFT vector.

254

255 *Confirmation of genetic transformation of plantlets*

256 Approximately 100 mg of young leaves obtained from putative transgenic plants were  
257 used to detect the integration of *neomycin phosphotransferase II (nptII)*, *GFP* and *RoFT*  
258 (DH primers) transgenes. DNA extraction was performed using Macherey-Nagel  
259 Nucleospin® Plant II.

260 PCR amplification was performed in a 10 µL volume containing 1X Taq buffer, 0.15 mM  
261 of each deoxyribonucleotide triphosphate (Promega), 2 mM MgCl<sub>2</sub>, 0.15 µM of each  
262 primer, 0.5 unit of Taq DNA polymerase (Promega) under standard PCR conditions [94°C  
263 2 min, (94°C 30 s, annealing temperature 15 s, 72°C 30 s) for 35 cycles, 72°C 7 min]. The  
264 primers chosen in different exons enabled amplification of the endogenous gene and the  
265 transgene with different PCR fragment sizes (1100 bp for endogenous gene and 536 bp  
266 for cDNA transgene; Fig. S1) and for the presence of *GFP* and *nptII* (data not shown). The  
267 primers are described in Table S1.

268

269 **RNA extraction and qRT-PCR**

270 For RNA analysis, cutting of 3 independent transgenic events were grown for GS  
271 (8.1\_1.1, 8.1\_1.2 and 8.2\_4.1) and PIMP (A2.1, C3.1 and C5.1). The leaves and dormant  
272 axillary buds were put under floral meristem on the plants after cold homogenization (see  
273 § Somatic embryo formation, multiplication, germination and plantlet formation) omitting  
274 the buds at the base of the stem.

275 Total RNAs were extracted from leaves and axillary buds using the NucleoSpin RNA Plus  
276 and RNA Plus XS kits, respectively (Macherey-Nagel, Düren, Germany). The absence of  
277 genomic DNA contamination was checked by PCR on total RNA. cDNAs were obtained by  
278 reverse transcription performed on 500 ng of total RNA using iScript Ready-to-use (Bio-  
279 Rad, Inc., Hercules, CA, USA). Real-time amplification (q-PCR) was performed with  
280 SsoADV Univer SYBR Green Supermix (Bio-Rad, Inc., Hercules, CA, USA) using cDNA as  
281 template, with the following program: 98°C for 30 s and 40 cycles (98°C for 10 s, 60°C for  
282 10 s). Fluorescence detection was performed using a CFX Connect™ Real-Time System  
283 (Bio-Rad, Inc., Hercules, CA, USA). The amount of cDNA per sample was normalized using

284 the *TCTP* (Randoux et al. 2012) and *UBC* (Klie and Debener 2011) genes, and the relative  
285 expression level was calculated according to Pfaffl (Pfaffl 2001) from three technical  
286 replicates per event. For RC2G0490100 (*Agamous D1*) and RC4G0415400 (*EuApetala 1 -*  
287 *CAULIFLOWER*) genes, primers were newly designed in the last exon and in the beginning  
288 (first 100 bp) of the 3'UTR using Primer3Plus (Untergasser et al. 2007). All primers are  
289 listed in Table S1. Data collection was performed using Bio-Rad CFX Maestro 1.1  
290 software.

291

### 292 **Phenotypic analysis**

293 Several measurements were obtained to monitor the effects of the transgene on the  
294 plant phenotype. The plants were grown in a confined greenhouse (type 2 according to  
295 French regulations) after *in vitro* multiplication and rooting. To homogenize the  
296 transgenic and non-transgenic plants, we cultivated them until the first flowering stage  
297 and then pruned all the plants and left them for 6 weeks at 4°C. The plants were then  
298 maintained in the greenhouse and gradually scored. The architectural data corresponded  
299 to the length and diameter of three to five floral stems, the length and number of  
300 internodes. For the floral organs, we counted all floral organs, i.e. sepals, petals, carpels  
301 and stamens for at least three different flowers per plant. The flowering ~~date-time~~  
302 corresponds to the number of days between the exit from the cold room and the first  
303 flower on the plant ~~flowering date~~. The first flower ~~flowering date~~ corresponds to the onset  
304 of flower development stage 4 described by Bergougnoux et al. (2007).

305

### 306 **Statistical analyses**

307 All data were statistically analyzed with the R software package, version 4.0.3 (2020-  
308 10-10) (<http://www.r-project.org/>), including ANOVA, HSD tests for multiple comparisons  
309 and T test.

310 The results are presented as a histogram (for qRT-PCR) and box-wisker plot for which  
311 the box covers 50% of the data, the vertical line that split the box is the median and the  
312 whiskers are the two lines outside the box, that go from the minimum to the lower quartile  
313 (the start of the box) and then from the upper quartile (the end of the box) to the maximum.

314

315 **Results**316 **RoFT gene family and phylogenetic analysis**

317 PEBP is a small eucaryot gene family. Using the *Rosa chinensis* reference genome  
 318 (Hibrand Saint-Oyant et al. 2018)–, we were able to detect six genes **encoding PEBP**  
 319 (RC4G04226500, RC3G0419200, RC6G0343500, RC1G0537200, RC7G0018600,  
 320 RC5G0373700). Note that one member of the family, i.e. *RoKSN* (HQ174211), the gene  
 321 controlling recurrent blooming (Iwata et al. 2011), is not present in the reference genome  
 322 as it has a *null* allele for this locus in the reference genome (Hibrand Saint-Oyant et al.  
 323 2018). This gene was included in the analysis. For the phylogenetic analysis, we added  
 324 *Arabidopsis A. thaliana* proteins from the PEBP family and annotated proteins from *Malus*  
 325 *domestica*, *Prunus persica* and *Fragaria vesca*. According to the phylogenetic tree (Fig.  
 326 1A), the ~~seven~~ rose PEBP genes ~~were~~ grouped into four clades. ~~T (TFL1-like, FT-like, BFT-~~  
 327 ~~like and MFT-like). Within the TFL1-clade contains s, two sub-clades (ATC and TFL1) were~~  
 328 ~~present, with RC5G0373700 and RoKSN as rose representatives respectively.~~  
 329 RC5G0373700 was previously denominated *RoTFL1* (Remay et al. 2009), based on our  
 330 new analysis we suggest renaming this gene *RoATCTFL1*. ~~RC7G0018600 was a member~~  
 331 ~~of the MFT clade which contained a single gene of each species. Two rose proteins~~  
 332 ~~(RC6G0343500 and RC2G0537200) belonged to the BFT clade. Concerning theThe~~ FT  
 333 clade ~~contains , two rose proteins were present~~ (RC3G0419200 and RC4G0426500,  
 334 previously denominated *RoFT* (Remay et al. 2009)) ~~whereas only one protein was present~~  
 335 ~~in apple and peach and 3 in woodland strawberry.~~ To go further in the *FT* analysis, we  
 336 have performed a micro-synteny analysis (Fig. 1B and C). A clear micro-synteny can be  
 337 demonstrated for the four genes (RC3G0419200, FvH4\_6g00090.1, Prupe.6G364900.1  
 338 and M12G1262000, Fig. 1B) that can be grouped into a subclade (Fig. 1A).  
 339 Another micro-synteny was found between a rose (RC4G0426500) and a strawberry  
 340 (FvH4-4g30710.1) gene, with no corresponding genes in *Prunus* and *Malus* (Fig. 1C). No  
 341 orthologous gene can be found for the third *Fragaria* gene. These results suggest that one  
 342 *FT* homologue exist in Rosaceae, with a diversification in Rosoid, with a duplication in  
 343 rose and strawberry. Another duplication (FvH4\_6g00090.1) also happened in *F. vesca*.

344 These results are in agreement with a recent analysis of *FT* diversification in Rosaceae  
 345 (Jiang et al. 2022).

346

### 347 **Choice of *RoFT* gene for in planta study and transformation results**

#### 348 *RoFT* gene

349 Among the *RoFT* genes, RC4G0426500 gene was previously proposed to be involved in  
 350 the control of various rose traits. The expression of this gene increases during spring and  
 351 is correlated with the flowering time of recurrent blooming (Remay et al. 2009) and non-  
 352 recurrent (Jiang et al. 2022) roses. Indeed, all inflorescence traits scored in a F1  
 353 population ('The Fairy' x hybrid of *Rosa wichurana*) co-localise on LG4, in the vicinity of  
 354 the *RoFT/RC4G0426500* locus (Iwata et al. 2011) and QTLs for flowering control have  
 355 been detected in this region in two cross-populations (Iwata et al. 2011; Roman et al.  
 356 2015). Moreover, the plant height and shape and some other architectural traits were  
 357 controlled by a QTL, that co-localised with the *RoFT* gene (Iwata et al. 2011). Moreover,  
 358 a study of architectural traits in rose showed that a QTL controlling the length of long  
 359 axes was located on linkage group 4 and co-localised with the *RoFT* gene (Li-Marchetti et  
 360 al. 2017). These genetic results suggest that *RoFT/RC4G0426500* may have a pleiotropic  
 361 role in flowering and plant architecture. To test this hypothesis, we opted to carry out an  
 362 *in planta* study on the overexpression of this *RoFT* gene located on LG4 (RC4G0426500).

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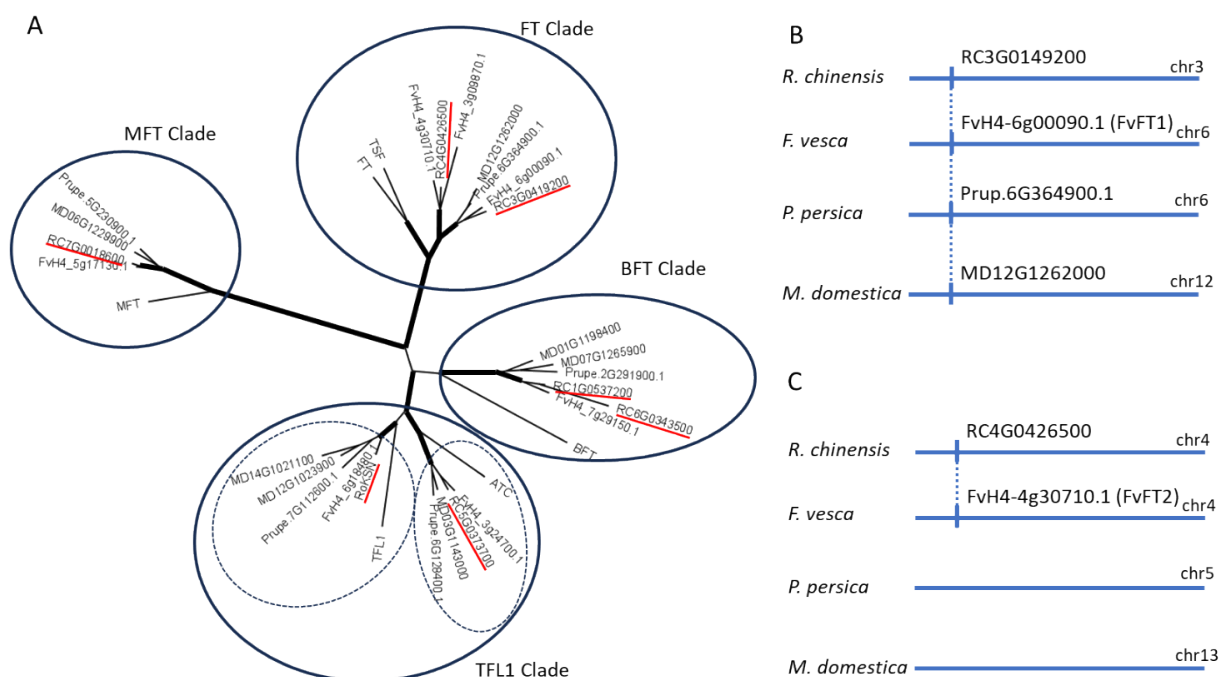
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376 **Fig. 1** PEBP family in rose (*R. chinensis*). **A.** Phylogenetic tree with the four clades (FT,  
 377 TFL1, MFT and BFT). The protein sequences were obtained from the GDR website  
 378 (<https://www.rosaceae.org/tools/jbrowse>) for rose (*Rosa chinensis* Genome v1.0), *Fragaria*  
 379 *vesca* (*F. vesca* Genome v4.0), *Malus domestica* (*Malus x domestica* GDDH13 v1.1) and  
 380 *Prunus persica* (*Prunus persica* Genome v2.0.a1). For the other proteins, the references  
 381 are: RoKSN(HQ174211), FT (AT1G65480), TSF (AT4G20370), TFL1 (AT5G03840), ATC  
 382 (AT2G27550), BFT (AT5G62040) and MFT (AT1G18100). Lines in red represent lines with  
 383 a bootstrap value > 80 (out of 100). Thick lines represent lines with a bootstrap value >  
 384 80 (out of 100). Rose proteins are underlined in red. **B.** and **C.** Microsynteny analysis for  
 385 the FT proteins within the FT clades. The synteny analysis was performed using the  
 386 syntenyviewer tools from GDR (<https://www.rosaceae.org/synview/search>). The syntenic  
 387 blocks are described in Table S1. No microsynteny was found for FvH4\_3g09870.1

### 388 *Transformation results*

389 The genetic transformation was performed on two genotypes *Rosa* DELdog  
 390 'Pimprenelle®' (PIMP), and *Rosa* DELtrimen 'Guy Savoy®' (GS). A flow cytometry study  
 391 showed that both these genotypes are tetraploid (2n=4X) (~~data not shown~~Fig. S1) and  
 392 have a different genotype for the *RoKSN* gene. GS has three alleles corresponding to  
 393 *RoKSN<sup>copia</sup>*, *RoKSN<sup>G181</sup>* and *RoKSN<sup>null</sup>* alleles while PIMP has only *RoKSN<sup>copia</sup>* and  
 394 *RoKSN<sup>G181</sup>* alleles (Fig. S2). The *RoKSN* alleles were denominated according to Soufflet-  
 395 Freslon et al. (2021). Both genotypes are described as continuous-flowering. ~~Somatic~~  
 396 ~~embryo clusters were used as target tissues (Fig. 2A) for genetic transformation of PIMP~~  
 397 ~~and GS.~~ The time to obtain ~~transformed and~~ regenerated *in vitro* cultured plants was  
 398 around a year corresponding to embryo germination (Fig. 2B to 2F) (or regeneration of  
 399 plants emerging from embryos), multiplication and rooting (Fig. 2G-H). The transgene  
 400 presence was checked by GFP protein expression under UV light at different embryo  
 401 development stages (Fig. 2C, 2D, 2I) and by PCR. ~~The primers chosen in different exons~~  
 402 ~~enabled amplification of the endogenous gene and the transgene with different PCR~~  
 403 ~~fragment sizes (1100 bp for endogenous gene and 536 bp for cDNA transgene; Fig. S1)~~  
 404 ~~and for the presence of GFP and nptII (data not shown).~~ We obtained several  
 405 transformation events and chose a limited number of regenerants for the experiments.  
 406 Ten and four events were thus transferred to the greenhouse and studied in detail for GS  
 407 and PIMP, respectively.

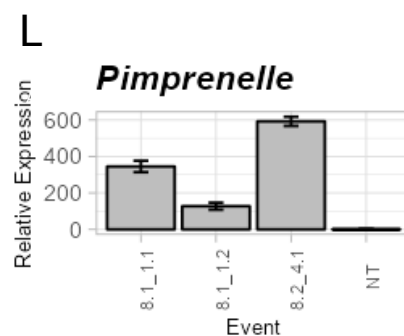
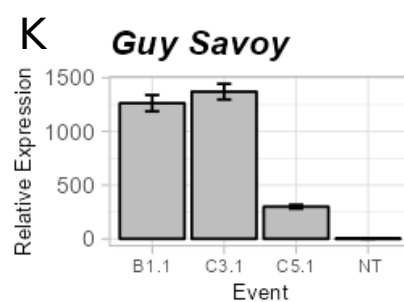
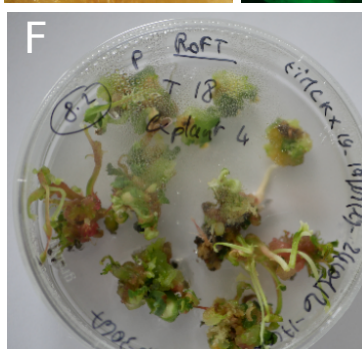
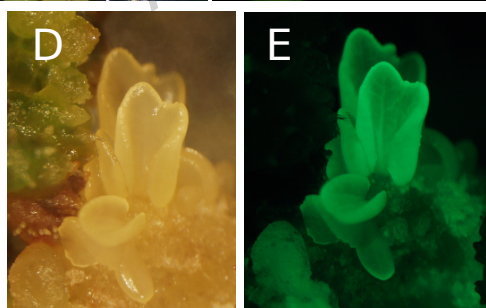
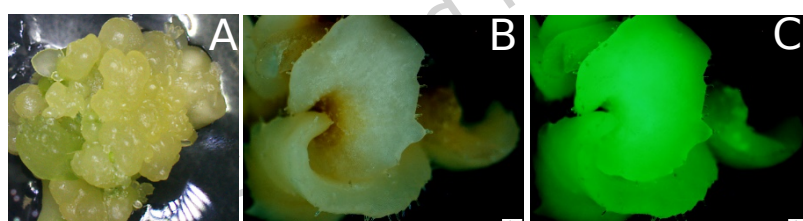
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### 409 **Phenotypic analysis of non-transformed and RoFT transformed plants**

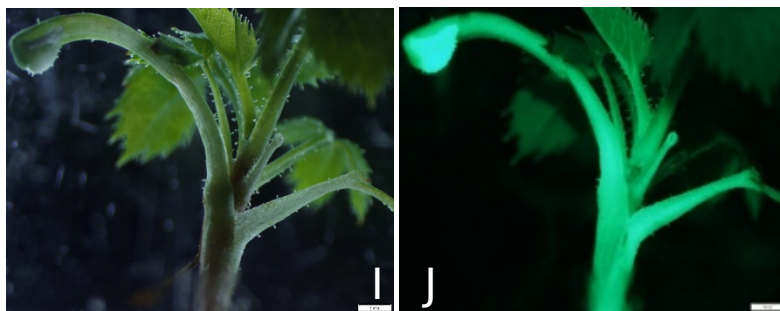


410 All transformation events of GS and PIMP were able to flower under the *in vitro*  
 411 conditions (Fig. 2G and 2H), except for one GS event C5.1. The *RoFT* transcript levels  
 412 were evaluated to compare the expression of three transgenic plants to that of the non-  
 413 transgenic plants (Fig. 2K and 2L). In the three GS transgenic events, on average the  
 414 *RoFT* transcript level had significantly >1200-fold and >1300-fold higher B1.1 and C3.1  
 415 events, respectively, than NT plants but this increase was not as marked for GS C5.1 (300-  
 416 fold). In PIMP, *RoFT* transcript accumulation was higher in the three transgenic events  
 417 compared to NT plants (130- to 600-fold higher transcript accumulation). *In vitro*-rooted  
 418 transgenic and non-transgenic (NT) plants were transferred to the S2 greenhouse. Similar  
 419 results were found in buds of GS (Fig. 6) and PIMP (Fig. 7)

420 The plants were scored for different traits after pruning and cold homogenization (see  
 421 Materials and Methods).



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**Fig. 2** Various rose genetic transformation steps. A) Somatic embryo clusters of *Rosa* DELdog Pimprenelle® genotype. B-E) Transformed somatic embryo of *Rosa* DELdog Pimprenelle® genotype 16 weeks after inoculation under white (B-D) and UV light (C-E). F) *in vitro* *Rosa* DELdog Pimprenelle® genotype regenerated transformed plantlets on kanamycine medium. G-H) *in vitro* rooted and blooming transformed plantlets of *Rosa* DELdog Pimprenelle® and *Rosa* DELtrimen Guy Savoy®. I-J) *in vitro* *Rosa* DELdog Pimprenelle® genotype regenerated transformed plantlets under white (I) and UV light (J). K-L) qRT-PCR was used to determine the relative expression levels of *RoFT* gene in leaves of transgenic and non-transgenic (NT) *Rosa* DELtrimen Guy Savoy® and *Rosa* DELdog Pimprenelle®. Results were expressed as mean of technical replicates  $\pm$  SD ( $n = 3$ ).

#### 459 *Flowering datetime*

460 Concerning the flowering *datetime* (Fig. 3) for GS genotype, 9 out of 10 transgenic  
461 events featured early flowering (40-50 days) compared to NT plants (57-97 days).

462 Flowering in one *RoFT*transgenic plant (C5.1) was significantly delayed (140-157 days)  
463 compared to NT plants. For PIMP, all *RoFT* events flowered earlier than NT plants, and  
464 three events (8.1\_1.1, 8.1\_1.2, 8.1\_4.1) showed significant differences compared to NT  
465 plants (12-17 days compared to 35-49 days for NT plants). The 8.1\_2.1 PIMP event  
466 flowered 28 days after a cold period but this flowering pattern did not appear to  
467 significantly differ from that of NT. In conclusion, *RoFT* overexpression can induce early  
468 flowering, but this effect is variable depending on the genotype and events within the  
469 same genotype.

470

#### 471 *Number of floral organs*

472 Figure 4 shows the number of petals, stamens, carpels and the total number of floral  
473 organs. The number of sepals, was always five for all transgenic and non-transgenic plants  
474 (not shown).

475 For GS *RoFT* events, no significant difference was observed in the petal number. For  
476 stamens, carpels and the total number of floral organs, significant differences were  
477 observed for seven transgenic events as compared to NT plants, while there was no  
478 significant difference for three events (A2.1, A2.2, C5.1) (Fig. 4A and Table S2). For the  
479 significantly different *RoFT* events, we noted a decrease in the number of stamens (50-  
480 112) compared to NT (82-171). Moreover, the carpel number ranged from 12 to 56 in  
481 these *RoFT* events compared to 46 to 85 for NT plants. As a result of the decreased stamen  
482 and carpel numbers, we observed a significant decrease in the total number of floral  
483 organs on those seven transgenic plants.

484 For the four transformed PIMP *RoFT* events, we found that *RoFT* overexpression  
485 significantly affected (Fig. 4B and Table S2) the petal and stamen numbers. Thus, an  
486 increase in the number of petals (10-25) compared to NT plants (5-9) and a reduction in  
487 the stamen number (48-96 vs 80-118) were observed. There was no significant difference  
488 between transgenic and non-transgenic plants with regard to the number of carpels and  
489 the total number of organs.

490

#### 491 *Architecture*

492 The plant architecture was evaluated by scoring the shoot size and diameter, and the  
493 internode number and size (Fig. 5 and Table S2) when the floral bud is visible. Concerning  
494 the stem diameter, ~~n~~No differences in stem diameter were observed between transgenic  
495 and non-transgenic plants in all genotypes and events (data not shown).

496 For the GS shoot size and internode number, there were no significances noted for  
497 three and two events, respectively (B1.1, A2.1, C5.1 and A2.1, C5.1) (Fig. 5 and Table S2).  
498 For the other events, the shoot size was significantly lower (10-31 cm) than in NT plants  
499 (38-70 cm). Moreover, the number of internodes in these events was lower (4-10)  
500 compared to 11-16 for NT plants. These observations were correlated with the earlier  
501 flowering ~~dates-times~~ of these events (Fig. 5 and Table S2), except for the C5.1 event.  
502 Concerning the mean internode number, no major differences were noted, except for one  
503 event (C3\_1) where there was a significant difference in comparison to NT plants.

504 For the PIMP genotype, a significant difference in the shoot size and internode  
505 number was observed for all *RoFT* events compared to NT plants. The mean shoot size  
506 was 10.8 cm in *RoFT* transgenic plants (5-18 cm), while that for the non-transformed  
507 plants was 35.33 cm (23-49 cm). These results were correlated with the early flowering  
508 observed in the *RoFT* plants (Fig. 3). Similarly, the internode number ranged from 3 to  
509 13, with a mean of 7.6, while the mean internode number was 15.66 (11-18) in NT plants  
510 (Fig. 4). No significant differences were observed in the mean internode size (Fig. 4).

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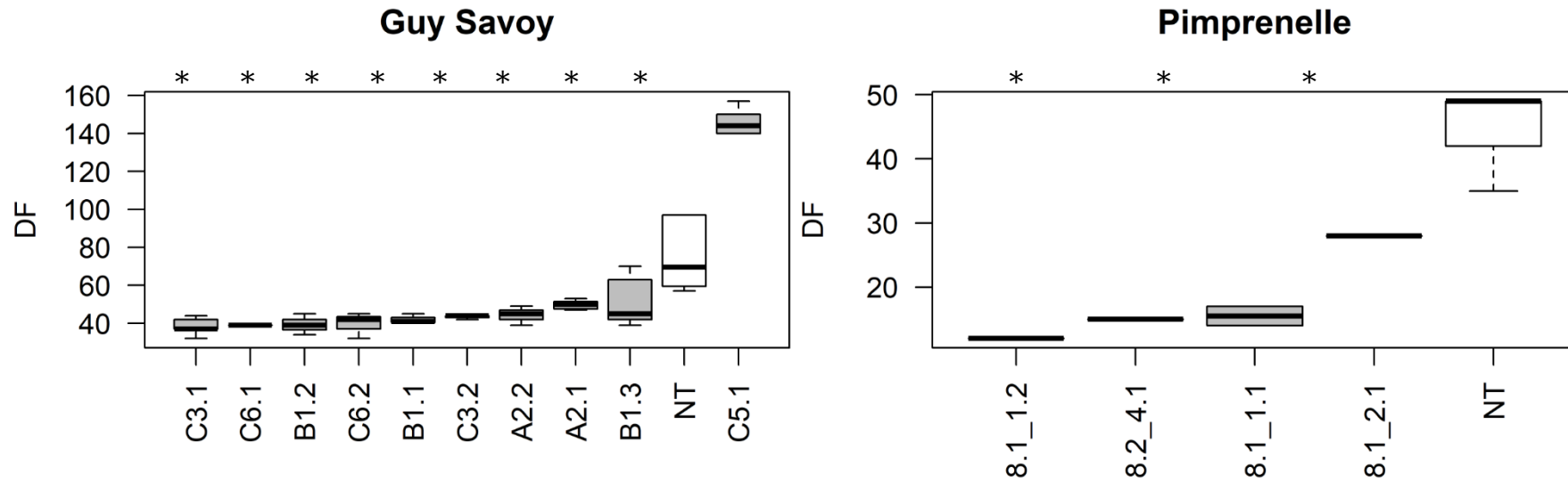
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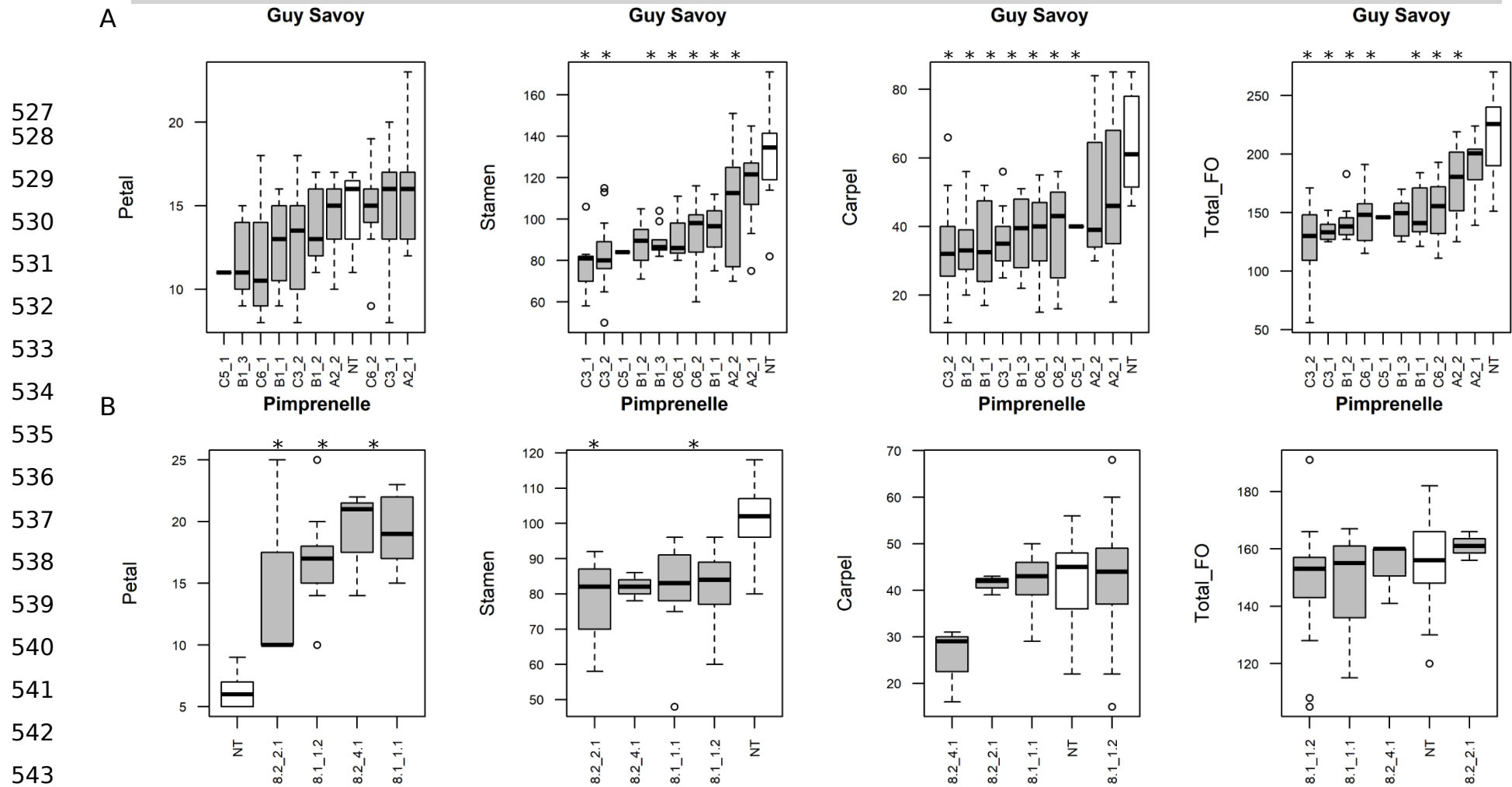
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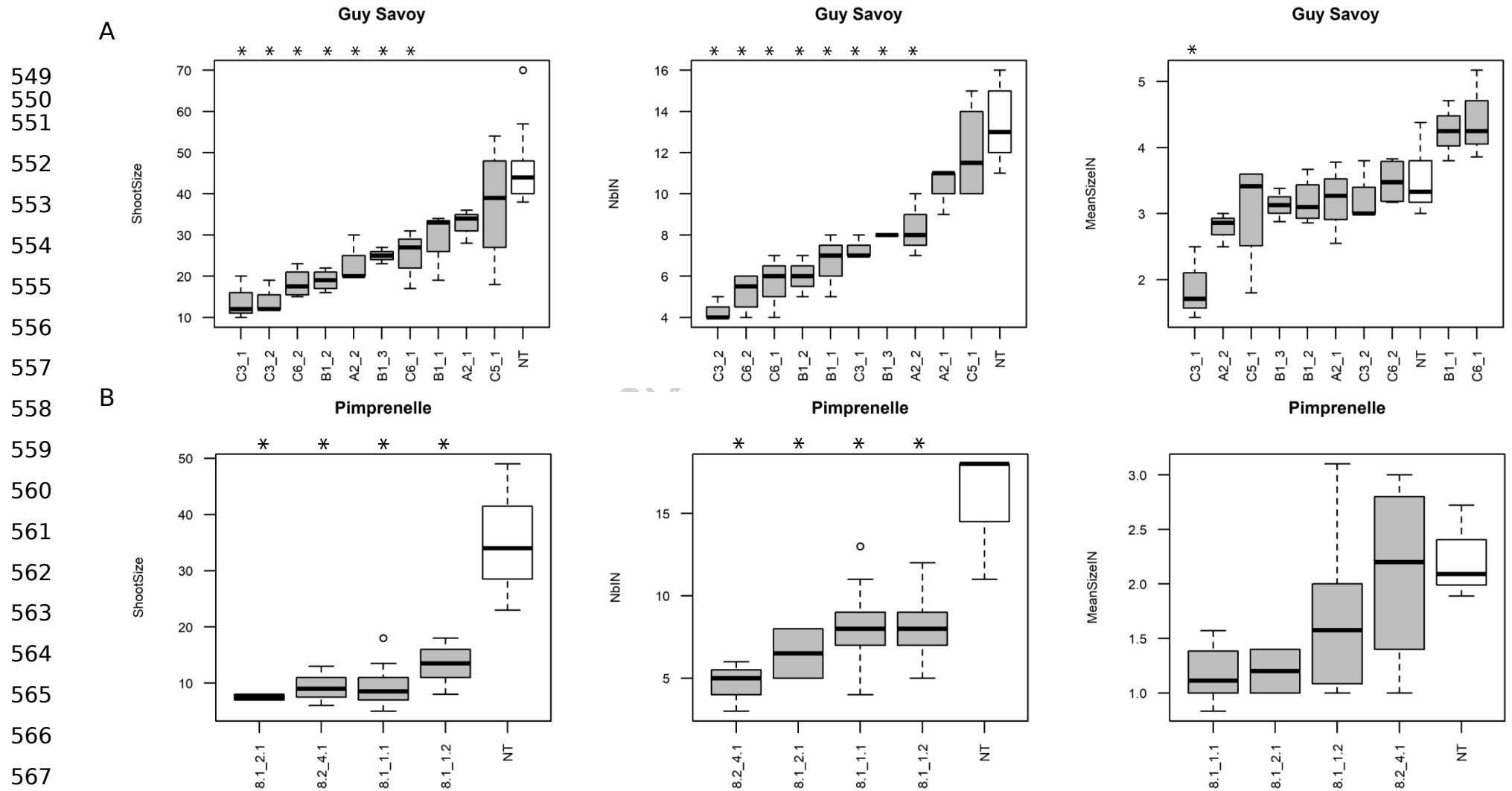
**Fig. 3** Flowering ~~date-time~~ in transgenic plants (in grey) and non-transformed plants (NT in white) for *Rosa DELtrimen* Guy Savoy® and *Rosa DELdog* Pimprenelle®. The mean flowering ~~dates-times~~ for three primary stems (DF) are indicated.

DF: ~~date-of-flowering-time~~, which corresponds, to the number of days between the plants coming out of the cold and the appearance of the flower (see Material and Methods).

\*= statistically significant difference from Tukey's multiple comparison test (Table S2).



544 **Fig. 4** Number of floral organs in transgenic plants (in grey) and non-transformed plants (NT in white) for *Rosa* DELtrimen Guy Savoy®  
 545 (*A*), *Rosa* DELdog Pimprenelle® (*B*). The mean of the number of petals (Petal), stamens (Stamen), carpels (Carpel) and the mean of total  
 546 number of floral organs (Total\_FO) are indicated. The number of counted flowers is for GS: NT [8], A2.1[11] A2.2[20], B1.1[8], B1.2[8],  
 547 B1.3[13], C3.1[9], C3.2[25], C5.1[5], C6.1[10], C6.2[10] and for PIMP: NT[28], 8.1\_1.1[13], 8.1\_1.2[25], 8.1\_2.1[3], 8.2\_4.1[3]. \*=  
 548 *statistically significant difference from Tukey's multiple comparison test (Table S3).*



568 **Fig. 5** Architecture of transgenic plants (in grey) and non-transformed plants (NT in white) for *Rosa* DELtrimen Guy Savoy® (A) and *Rosa*  
 569 DELdog Pimprenelle® (B). The mean the shoot size (ShootSize), the internode number (NbIN) and the mean of internode size (MeanSizeIN)  
 570 are indicated. The number of counted stems is for GS: NT [9], A2.1[3] A2.2[3], B1.1[3], B1.2[4], B1.3[3], C3.1[3], C3.2[3], C5.1[4], C6.1[3],  
 571 C6.2[4] and for PIMP: NT[3], 8.1\_1.1[10], 8.1\_1.2[10], 8.1\_2.1[2], 8.2\_4.1[4].  
 572 \*= statistically significant difference from Tukey's multiple comparison test (Table S3).

573 *Floral gene expression analysis*

574 We then aimed to go further in the molecular characterization of the transgenic  
 575 plants, and to assess how *FT* overexpression could modify the expression of some key  
 576 floral genes. We thus studied the expression level of the target *FT* genes (rose homologues  
 577 of *SOC1*, *FRUITFULL* and *API1*), antagonist or interactive partners of *FT* genes (*RoATC*,  
 578 *RoKSN*, a *TFL1* homologue and *FD*) and genes controlling flower development (*AP1*, *AP2*,  
 579 *AP3* and *AGAMOUS*) in buds for three events per genotype, i.e. A2.1, C3.1 and C5.1 for  
 580 GS and 8.1\_1.1, 8.1\_1.2 and 8.2\_4.1 for PIMP.

581 Some of these events were chosen because they presented extremes in phenotypic  
 582 characters such as the shoot size for GS\_C3.1 and the flowering ~~date-time~~ delay for  
 583 GS\_C5.1, and the floral organ number (e.g. carpels for 8.2\_4.1) and architecture data for  
 584 PIMP events. For all genes studied, Figures 6 and 7 show the mean relative transcript  
 585 accumulation of transgenic and NT plants. In all the analyses, NT plants were chosen as  
 586 the reference (value of 1) in terms of the relative transcript accumulation.

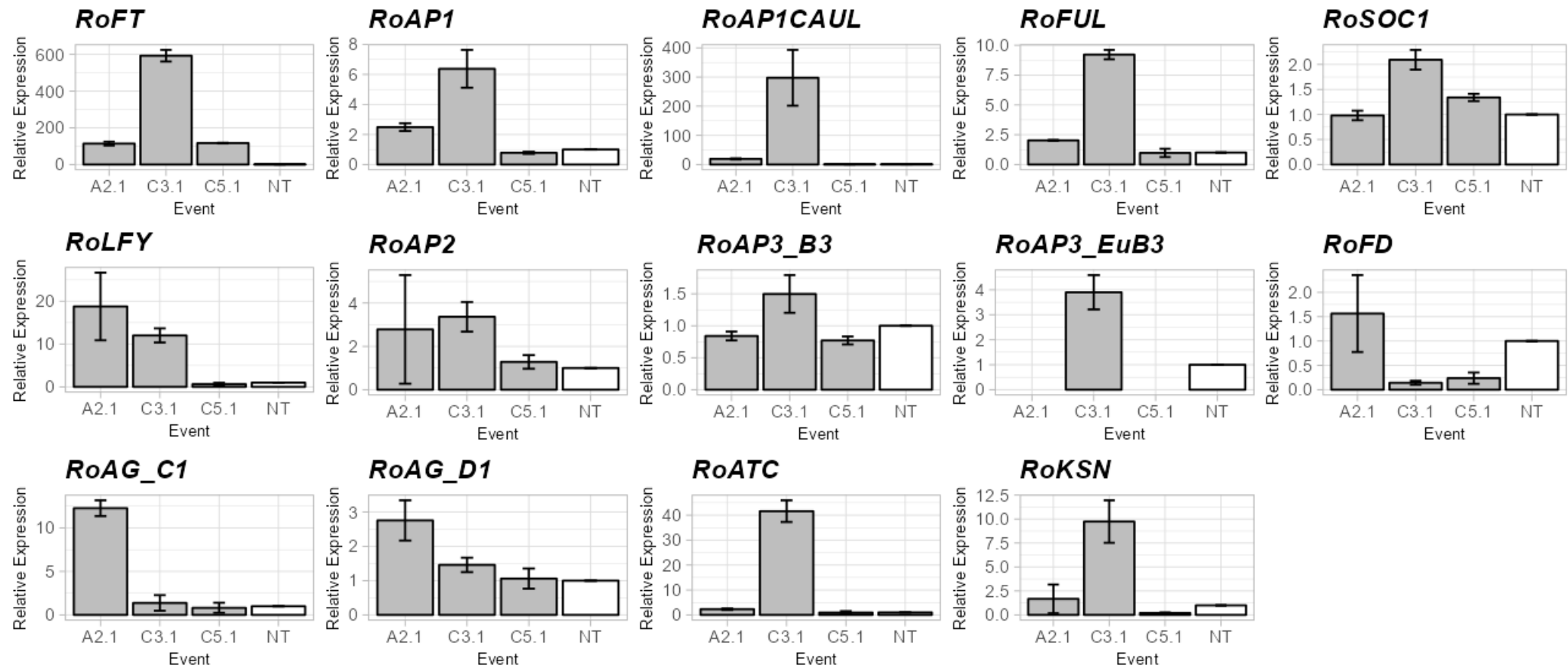
587 Higher *RoFT* transcript accumulation in buds was observed for transgenic GS and  
 588 PIMP plants compared to NT plants (110-fold to 1850-fold higher), and transcript  
 589 accumulation was higher in the PIMP *RoFT* genotype compared to the GS *RoFT* genotype.

590 Concerning the genes involved in floral initiation (*RoAPI1*, *RoAP1CAUL*, *RoFUL*,  
 591 *RoLFY*, *RoSOC1*) for PIMP and (*RoAPI1*, *RoAP1CAUL*, *RoFUL*, *RoLFY*), for GS (except  
 592 C5.1), we found that the transcripts were accumulated to a greater extent in transgenic  
 593 plants (Fig. 6). In GS, note that for these genes, transcript accumulation was higher in  
 594 C3.1 plants, that accumulate 600-fold higher *RoFT* transcript. Concerning *RoSOC1*, no  
 595 higher transcript accumulation was observed in GS A2.1, while the accumulation was  
 596 significant but very low in both other events. For GS C5.1, no higher accumulation was  
 597 observed for the floral initiation genes. Interestingly, for GS (except C5.1) and PIMP, we  
 598 also observed transcript accumulation of the *RoATC* floral repressor (with 40-fold  
 599 accumulation in some events). Concerning *RoKSN*, no variations were noted in PIMP,  
 600 whereas there was greater transcript accumulation in GS (except for C5.1 which showed  
 601 lower accumulation).

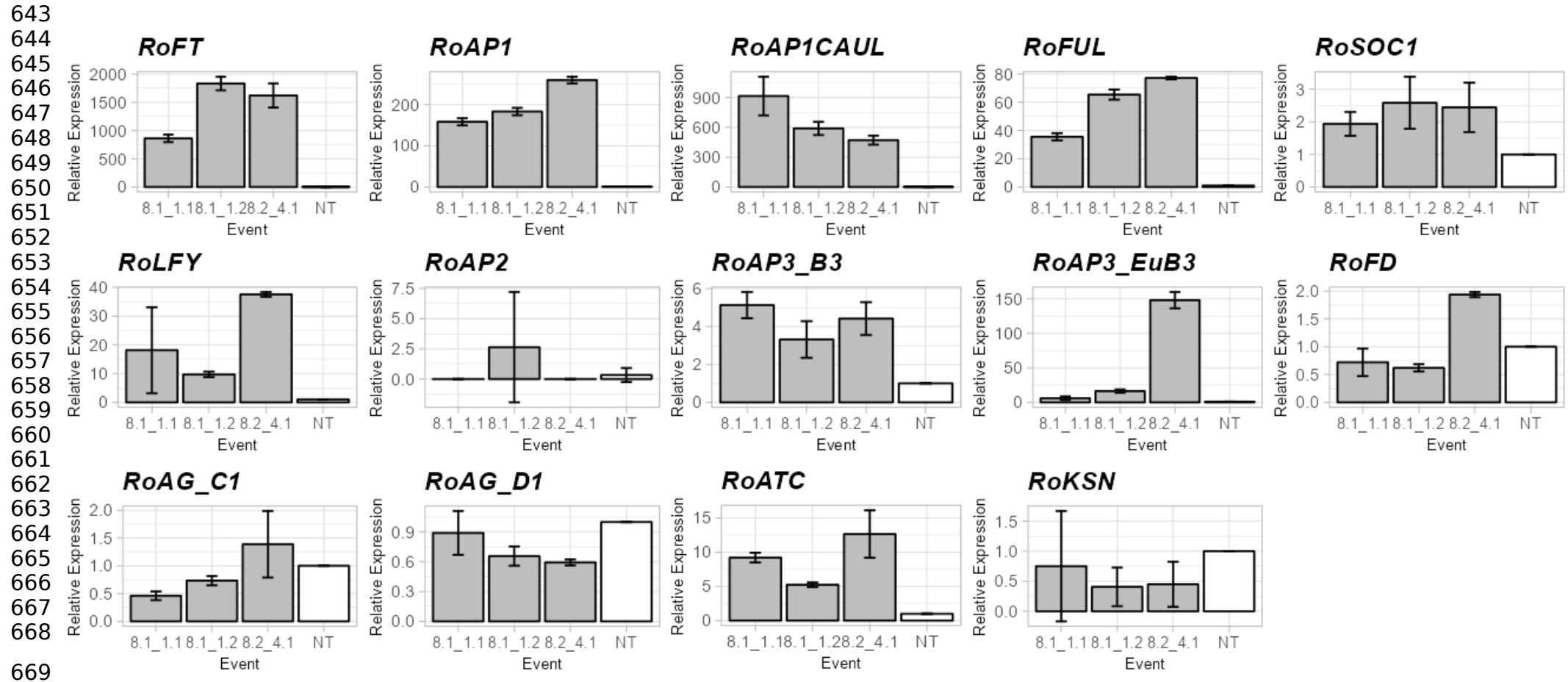


602 High variability was observed with regard to the floral identity genes. It should also  
603 be noted that the expression was studied in shoot axillary buds (and not in floral buds). In  
604 GS, upregulation was observed for *RoAG\_D1* and *RoAG\_C1* for A2.1, i.e. an event with no  
605 variation in floral organs (Fig. 4). Concerning PIMP, there was higher *AP3* transcript  
606 accumulation, and the same trends seem to apply in *AP2* (but marked variability was  
607 observed for this gene).

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**Fig. 6** Relative expression levels of flowering-related genes (genes of Class-ABC: *RoAP1*, *RoAP1\_CAUL*, *RoAP2*, *ROAP3\_B3*, *RoAP3\_euB3*, *RoAG-C1*, *RoAG-D1* and *RoFT*, *RoSOC1*, *RoFF(AP1/FRUITFULL)*, *RoKSN*, *RoATC*, *RoFD*, *ROLFY*) in buds of transgenic (in grey) and non-transgenic (NT in white) *Rosa DELtrimen Guy Savoy®* plants. Results were expressed as mean of technical replicates  $\pm$  SD ( $n = 3$ ).



670 **Fig. 7** Relative expression levels of flowering-related genes (genes of Class-ABC: *RoAP1*, *RoAP1\_CAUL*, *RoAP2*, *ROAP3\_B3*,  
671 *RoAP3\_euB3*, *RoAG-C1*, *RoAG-D1* and *RoFT*, *RoSOC1*, *RoFF(AP1/FRUITFULL)*, *RoKSN*, *RoATC*, *RoFD*, *ROLFY*) in buds of  
672 transgenic (in grey) and non-transgenic (NT in white) *Rosa DELdog Pimprenelle*® plants. Results were expressed as mean of technical  
673 replicates  $\pm$  SD ( $n = 3$ ).

## 674 **Discussion**

675 *FT* genes encode a mobile protein that mediates numerous developmental  
676 processes such as growth, plant architecture control, fruit set and tuber formation (Pin  
677 and Nilsson 2012).

678

### 679 **Seven member genes of the rose PEBP family**

680 In this study, we analysed the PEPB family in the rose genome and characterized  
681 the seven PEBP members and grouped them in four clades (FT, TFL1, MFT and BFT), with  
682 each containing at least one *Arabidopsis* PEBP gene. In contrast, Zhao et al. (2020) only  
683 found three clades (FT, TFL1 and MFT) in 24 PEBP genes in pear. There are two rose  
684 members within the FT clade (RC3G0419200 and RC4G04226500, respectively located  
685 on chromosomes 3 and 4 (Hibrand Saint-Oyant et al. 2018)), which correspond to the  
686 FvFT1 and FvFT2 respectively (phylogenetic and micro-synteny analysis). We selected the  
687 FT member RC4G04226500 based on the findings of previous studies that highlighted a  
688 correlation of this gene with different flowering and architecture QTLs (Iwata et al. 2011;  
689 Li-Marchetti et al. 2017; Remay et al. 2009). This gene was previously found to be a floral  
690 activator based on its overexpression in *Arabidopsis thaliana*, with an early flowering  
691 phenotype, (Otagaki et al. 2015; Randoux et al. 2014). We decided to study the function  
692 of this *FT* homologue by producing rose that ectopically expressed this gene. We have  
693 characterized the transgenic plants at the phenotypic and molecular level.

694

### 695 **High variability in the rose transformation process**

696 In many studies, FT was functionally validated in a heterologous recipient system  
697 such as *Arabidopsis thaliana*, which favours easy and quick genetic transformation  
698 (Koembuoy et al. 2020; Tanaka et al. 2014; Zhang et al. 2010; Zhi-Yi et al. 2020). The  
699 RoFT homologue studied in this study has been previously demonstrated to be a floral  
700 activator in rose by complement of a *ft* mutant in *Arabidopsis* (Randoux et al. 2014). In  
701 this study, we decided to focus on rose for genetic transformation as this process is  
702 generally long and inefficient. Rose is considered to be recalcitrant to regeneration  
703 (Nguyen et al. 2017) although this is a crucial step in the genetic transformation process,

704 which in turn may be highly affected by the genotype. Indeed, there have only been 20  
705 papers published on stable genetic transformation in rose and only one genotype was  
706 transformed in most of these studies.

707

### 708 **Inter- and intra-genotype variability in RoFT transgenic plants**

709 Here we obtained several *RoFT* overexpression events for the two genotypes, i.e.  
710 PIMP and GS. Variations in phenotypic characters and gene expression were noted  
711 between and within the two genotypes.

712 First, we observed a difference in *RoFT* transcript accumulation in transgenic  
713 plants, i.e. between events and genotypes, and between leaves and buds. High transgene  
714 expression variability in independent transformants has been frequently reported (Butaye  
715 et al. 2004; Day et al. 2000; Kirchhoff et al. 2020; Peach and Velten 1991; Rosin et al.  
716 2008). Several hypotheses and explanations have been put forward to explain these  
717 differences. First, natural variation can arise spontaneously (Anderson et al. 2016) during  
718 the tissue culture process (Lambirth et al. 2015; Neelakandan and Wang 2012). Indeed,  
719 somaclonal variation is known in *Rosa* species (Arene et al. 1993), and variations in  
720 methylation profiles has been observed in rose during somatic embryogenesis and *in vitro*  
721 organogenesis (Xu et al. 2004).

722 Secondly, during the transformation process, variability arise due to the transgene  
723 number, position effects and epigenetic silencing.

724

### 725 **FT homologue overexpression in rose leads to early flowering**

726 In both PIMP and GS genotypes (except for C5.1, see below), we observed early  
727 flowering in transgenic roses (Fig. 3). In some cases, flowers were also observed under *in*  
728 *vitro* conditions. This phenotype was expected as FT is known to be a major floral activator  
729 (Kinoshita and Richter 2020) and similar results have also been obtained in other woody  
730 plants (Bohlenius et al. 2006; Hsu et al. 2006; Klocko et al. 2016; Tanaka et al. 2014;  
731 Wenzel et al. 2013). The ectopic expression of the orthologous gene in *F. vesca*, *FvHA-*  
732 *4g30710.1/FvFT2*, is also responsible of precocious flowering and dwarf plants (Gaston  
733 et al. 2021). The early flowering phenotype featured shorter shoots and fewer internodes,

734 whereas the internode length was not modified by *RoFT* overexpression (Fig. 5). This  
735 suggested that early flowering was due to early floral induction, leading to shoots  
736 producing fewer internodes. We sought to understand how overexpression of *RoFT* could  
737 lead to early floral induction by studying the expression of major genes involved in floral  
738 initiation. In all transgenic events (except C5.1), we observed higher transcript  
739 accumulation of floral initiation genes, such as homologues of *SOC1*, *AP1*, *FUL* or *LFY*.  
740 Surprisingly, floral repressor (*RoATC*) transcripts were also accumulated (Fig. 6). It was  
741 previously shown in rose that ectopic expression of a floral repressor from the same family  
742 (*RoKSN*) led to non-flowering of continuous-flowering plants with high repression of floral  
743 initiation genes such as *LFY* or *AP1* (Randoux et al. 2014). This *RoTFL1* transcript  
744 accumulation might be explained by a negative feedback loop to avoid to precocious  
745 flowering.

746         Concerning the C5.1 event in GS, we observed no *RoFT* accumulation in buds,  
747 whereas high accumulation was detected in leaves (Figs. 2 and 6). Moreover, associated  
748 with *RoFT* accumulation pattern, the C5.1 plants flowered later than the non-transgenic  
749 plants. This further suggested that transgene silencing was involved in this event. Since  
750 the first report of transgene silencing in petunia (Napoli et al. 1990), several studies have  
751 shown that the 35S transgene could be silenced (Fan et al. 2011; Jin and Guo 2015;  
752 Mishiba et al. 2005; Sohn et al. 2011). In agreement with this phenotype, we observed an  
753 absence of accumulation of the floral initiation gene transcripts (Fig. 6). Interestingly,  
754 despite the substantial flowering delay, C5.1 plants had the same number of internodes  
755 and the same shoot size as the non-transgenic plants (Fig. 5). Therefore, the flowering  
756 delay was not due to the production of more internodes.

757

### 758 **Variability in floral organs**

759         As expected, *FT* ectopic-expression impacted the flowering date, but more  
760 surprisingly it also affected floral development, particularly the number of different floral  
761 organs. *RoFT* transcripts (*RC4G0426500*) have been previously shown to be accumulated  
762 in floral bud (Remay et al, 2009; Jiang et al, 2021), suggested a possible role of this gene  
763 in flower development. In both genotypes (GS and PIMP), we observed a variation in floral

764 organs, but both genotypes responded differently. In GS, seven out of the ten studied  
765 transgenic plants presented fewer stamens and carpels, yet the petal and sepal numbers  
766 were not affected (Fig. 4). Consequently, the total number of floral organs decreased in  
767 these transgenic plants, suggesting mitotic rather than homeotic conversion. The A2.1  
768 event did not show any differences in the number of floral organs compared to NT plants  
769 and, despite the presence of *RoFT* in buds, and except for *RoLFY*, *RoFT* targets were  
770 weakly upregulated (*RoAP1*, *RoAP1CAUL*, *RoFF*). In this event, we also observed  
771 upregulation of *RoAG* genes without any phenotype linked to this activation. For the C3.1  
772 event, all *RoFT* targets were upregulated and we noted upregulation of *RoAP3\_euB3*. The  
773 role of this class B gene is still unclear, but Hibino et al. (2006) hypothesized that this  
774 gene is involved in petal and stamen development, and in our study the C3.1 event was  
775 the one with the least number of stamens. In this event, we also observed upregulation of  
776 *RoATC* and *RoKSN* (a TFL1-like gene family (Iwata et al. 2011)).

777 In PIMP, we observed more petals and fewer stamens in all of the transgenic plants. The  
778 number of sepals and carpels was not modified. Furthermore, the total number of floral  
779 organs was similar between transgenic and NT plants, suggesting that the stamens were  
780 in the process of converting into petals. This homeotic conversion has already been  
781 observed in rose, and is associated with the mis-regulation of ABCE model genes (Dubois  
782 et al. 2011). In such a scenario, it could be expected that A type genes would be more  
783 expressed while C type genes would be less expressed, leading to more petals and less  
784 stamens. However, we did not observe any significant variations in A class genes (*AP2*) or  
785 C class genes (*AG*). Only class B gene transcripts (*AP3\_B3*) were more accumulated. In  
786 pear, citrus *FT* overexpression induces a modification in the number of floral organs, while  
787 there is an increase in petal number in some events (Matsuda et al. 2009). Similarly,  
788 Tanaka et al. (2014) showed that *AtFT* transgenic apple lines contained more petals and  
789 fewer stamens than non-transformed apple cultivars, as was observed in the PIMP  
790 genotype. These lines feature *LEAFY* ortholog upregulation, which might affect the floral  
791 organ number and shape (Mimida et al. 2011).

792 In this study, we were able to transform two rose genotypes and showed that *RoFT* may  
793 have multiple functions in floral initiation and development. However, further research is

794 needed to gain further insight into how *RoFT* is involved in these mechanisms.  
795 Nevertheless, our findings demonstrated that rose transformation could serve as a model  
796 for molecular genetic analyses to elucidate the reproductive mechanisms of ornamental  
797 plants.

798

799 **Supplementary information** tables S1 to S4 and Figs. S1 and S2

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811 **Author contribution** FF, LH, LHSO conceived and managed this study and wrote this  
812 manuscript. LH, LV, JB, SA, SF managed *in vitro* plants, conducted plant genetic  
813 transformation. JC, TT conducted data collecting and managed the plants into the  
814 greenhouse. LO and JJ cloned *RoFT* for *Agrobacterium tumefaciens* genetic  
815 transformation. JB, LO conducted qRT-PCR experiments.

816 **Data available on request** The data underlying this article will be shared on reasonable  
817 request to the corresponding author.

818 **Declaration of interest** The authors declare that they have no conflicts of interest

819

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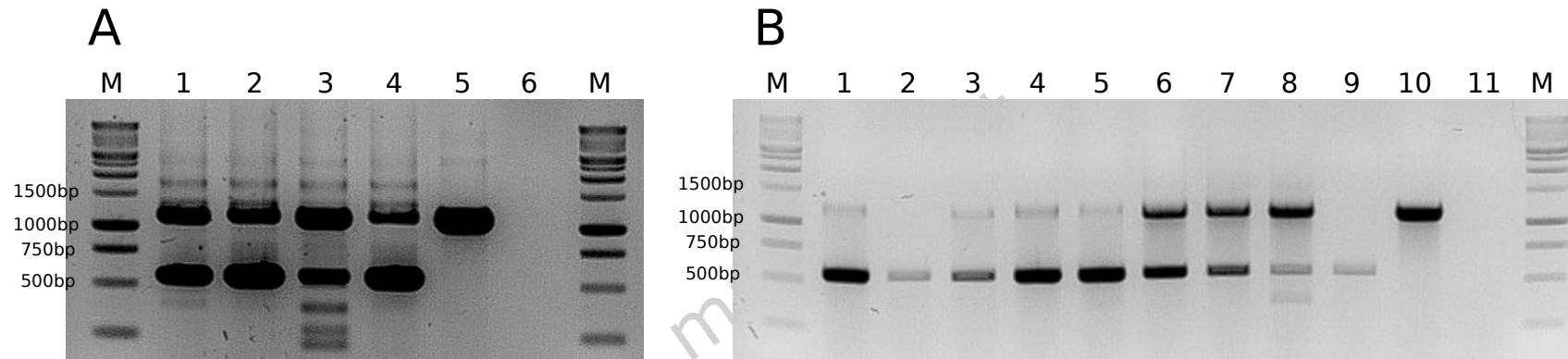
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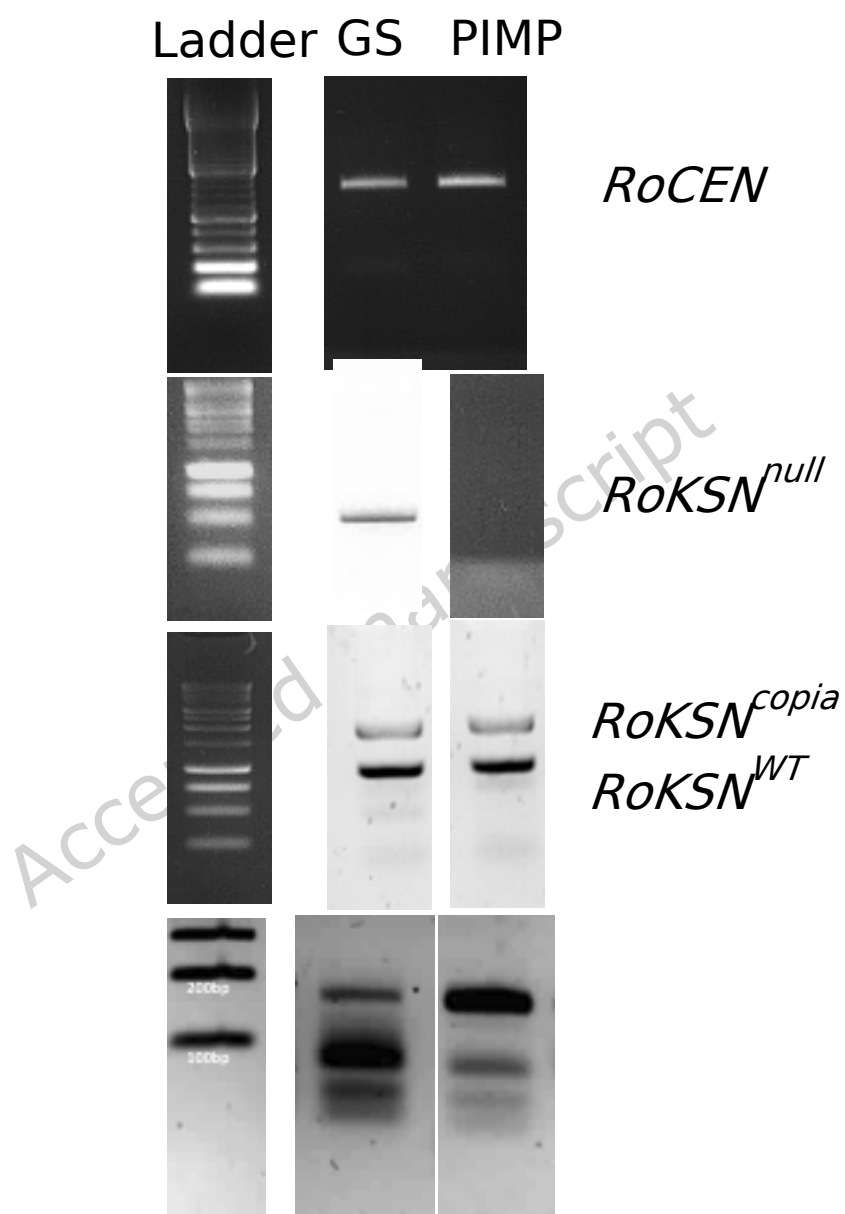
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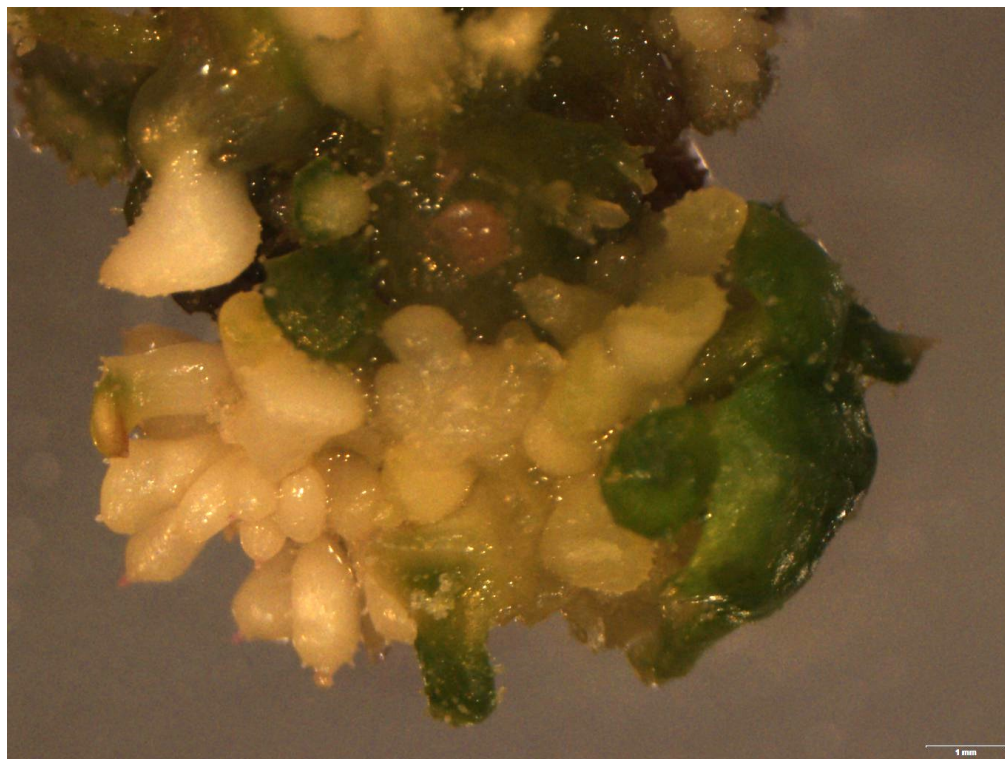


**Fig. S1.** Result of PCR amplifications. *RoFT* amplification of genomic DNA (1110 bp) and transgene cDNA (536 bp).  
 A: *Rosa* DELdog Pimprenelle®) : Lane 1, 8.1\_1.1, lane 2, 8.1\_1.2, lane 3 8.1\_1.2, lane 4, 8.2\_4.1, lane 5, NT, lane 6, negative control.  
 B: *Rosa* DELtrimen Guy Savoy®) : lane 1, A2.1 , lane 2, A2.2, lane 3 , B1.1, lane 4, B1.2, lane 5, B1.3, lane 6, C3.1, lane 7, C3.2, lane 8, C5.1, lane 9, C6.1, lane 10, NT, lane 11, negative control. M molecular weight ladder.

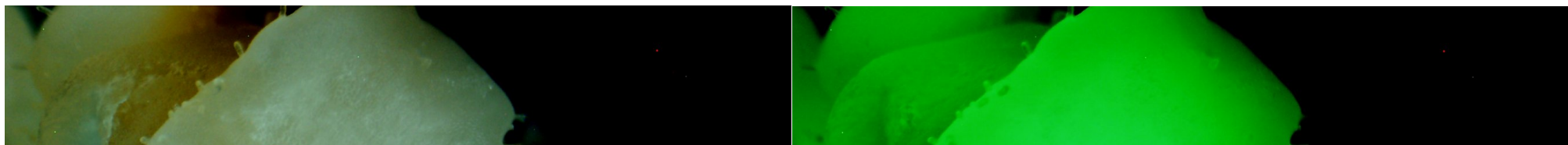


**Fig. S2:** Genotyping of *RoKSN* for GS and PIMP. Using different primers, we have genotyped all the known alleles at the *RoKSN* locus. The genotyping was done based on previous described methods for *RoKSN<sup>copia</sup>* (31), *RoKSN<sup>null</sup>* (33), *RoKSN<sup>A181</sup>* and *RoKSN<sup>G181</sup>* (34). GS is *RoKSN<sup>null</sup>* / *RoKSN<sup>copia</sup>* whereas PIMP is *RoKSN<sup>copia</sup>* / *RoKSN<sup>G181</sup>*. The presence of the *RoKSN<sup>G181</sup>* allele in GS is explained by the presence of the G at position 181 in the *RoKSN<sup>copia</sup>* allele.

Photo A



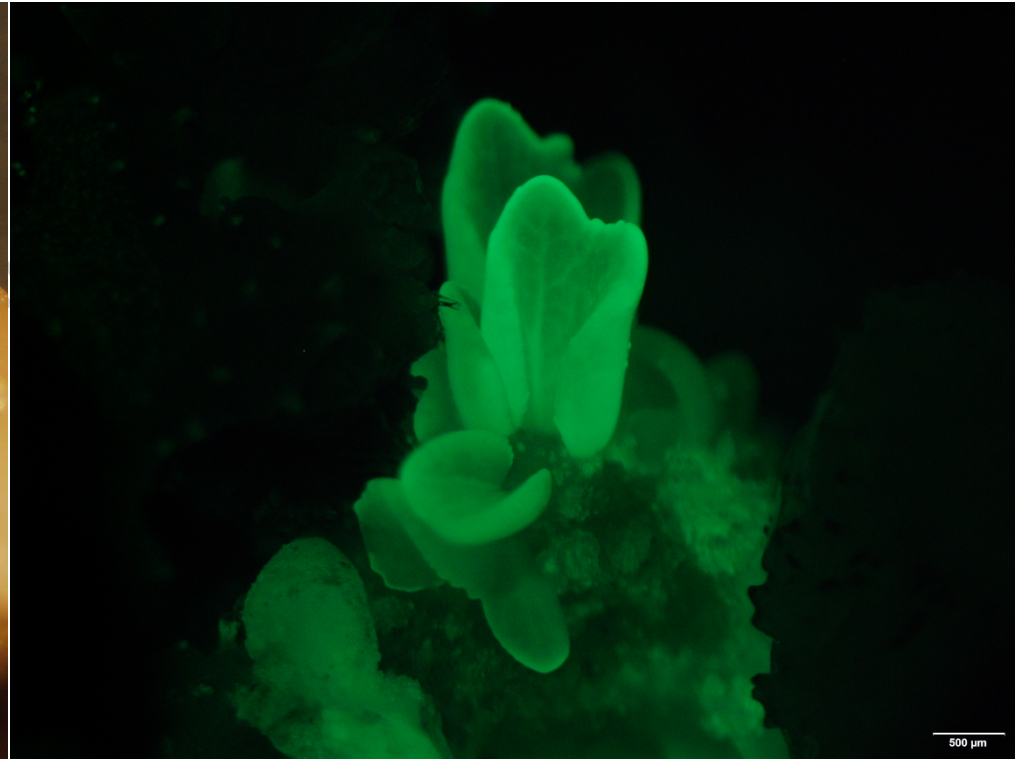
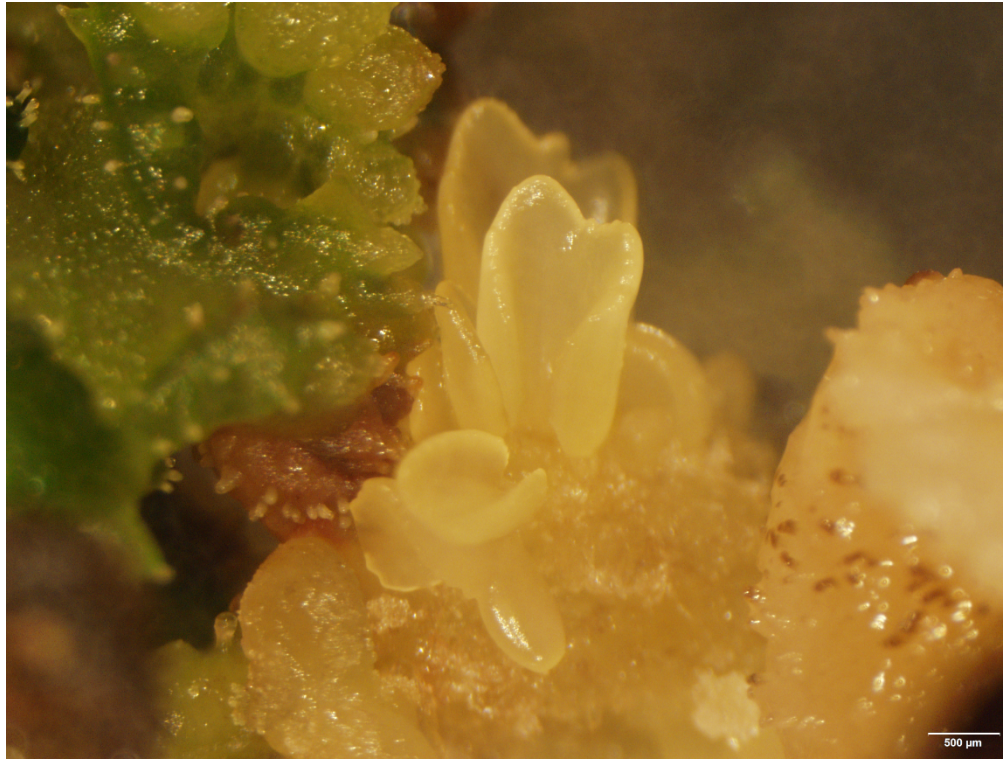
Photos B and C



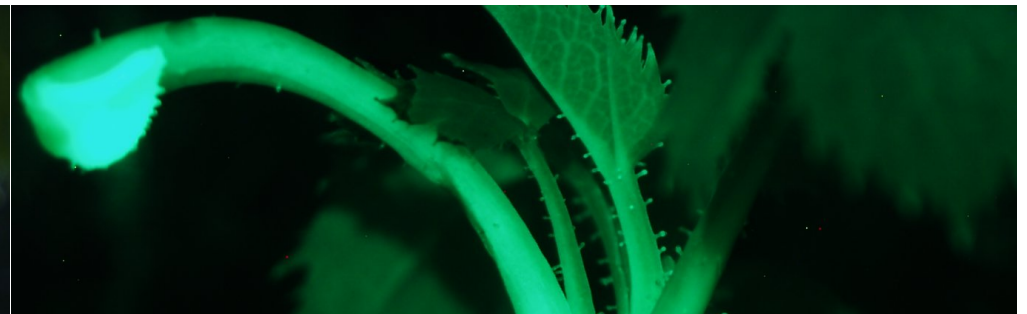
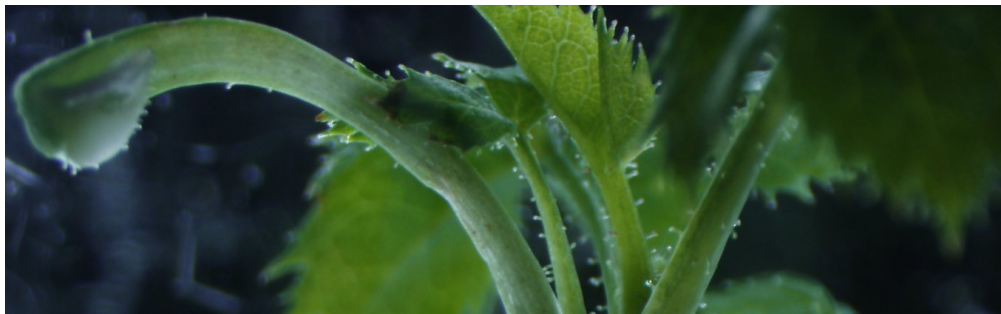


Accepted manuscript

Photos D and E



Photos I and J



Accepted manuscript

**Table S1.** Syntenic blocks. The synteny analysis was performed using the synteny viewer tool from the GDR (<https://www.rosaceae.org/synview/search>). The rose chromosomes 3 and 4 (location of RC3G0149200 and RC4G0426500) and the strawberry chromosome 3 (location of FcH4\_3g09870.1) were selected as genome and chromosome to search blocks by a given location against other *Rosaceae* genome: *Fragaria vesca* (*F. vesca* Genome v4.0), *Malus*

| Genes        | Syntenic blocks       |     |                       |     |                        |     |
|--------------|-----------------------|-----|-----------------------|-----|------------------------|-----|
|              | <i>Fragaria vesca</i> |     | <i>Prunus persica</i> |     | <i>Malus domestica</i> |     |
|              | Name                  | Chr | Name                  | Chr | Name                   | Chr |
| RC3G0149200  | ftrcB290              | 6   | pprcB303              | 6   | mdrcB183               | 12  |
| RC4G0426500  | ftrcB182              | 4   | pprcB277              | 5   | mdrcB218               | 13  |
| FvH3_3g09870 | -                     | -   | -                     | -   | -                      | -   |

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**Table S2.** Primers used for PCR and qRT-PCR analyses.  
RC number corresponds to the number of gene annotation in Hibrand-sain

|         | <b>Rose gene</b>  | <b>name</b>                               |
|---------|-------------------|---|
| class A | <i>RoAP1</i>      | EuApetala 1 - CAULIFLOWER                 |
| class A | <i>RoAP1</i>      | Apetala 1                                 |
|         | <i>RoFF</i>       | Apetala 1/FRUITFULL                       |
| class A | <i>RoAP2</i>      | Apetala2/TOE homologue                    |
| class B | <i>RoAP3_B3</i>   | Masako B3                                 |
| class B | <i>RoAP3_EuB3</i> | Masako EuB3                               |
| class C | <i>RoAG_C1</i>    | Agamous C1                                |
| class C | <i>RoAG_D1</i>    | Agamous D1                                |
|         | <i>RoFD</i>       | Flowering locus D                         |
|         | <i>RoFT</i>       | Flowering locus T                         |
|         | <i>RoLFY</i>      | Leafy                                     |
|         | <i>RoSOC1</i>     | Suppressor of overexpression of CO 1      |
|         | <i>RoTFL1</i>     | Terminal flower 1                         |
|         | <i>UBC</i>        | ubiquitin conjugating protein             |
|         | <i>TCTP</i>       | Translationally controlled tumour protein |
|         | <i>nptII</i>      | neomycin phosphotransferase II            |
|         | <i>GFP</i>        | green fluorescent protein                 |

at Oyant et al. 2018

| <b>located on<br/>Rose</b> | <b>RC number</b> |
|----------------------------|------------------|
| 4                          | RC4G0415400      |
| 7                          | RC7G0064200      |
| 2                          | RC2G0592900      |
| 3                          | RC3G0243000      |
| 2                          | RC2G0142200      |
| 6                          | RC6G0508500      |
| 5                          | RC5G0115100      |
| 2                          | RC2G0490100      |
| 6                          | RC6G0520900      |
| 4                          | RC4G0426500      |
| 7                          | RC7G0109100      |
| 1                          | RC1G0310700      |
| 5                          | RC5G0373700      |
| 7                          | RC7G0173600      |
| 4                          | RC4G0282100      |
|                            |                  |
|                            |                  |
|                            |                  |

| <b>function</b>  | <b>primer name</b>           |
|--|------------------------------|
| K-box region and MADS-box transcription factor family protein  | RoAP1CAUL_F<br>RoAP1CAUL_R   |
| AGAMOUS-like 8   | RoAP1_F<br>RoAP1_R           |
| AGAMOUS-LIKE 8, AGL8, FRUITFULL, FUL                           | RoFF_F<br>RoFF_R             |
|  | RoAP2_F<br>RoAP2_R           |
| K-box region and MADS-box transcription factor family protein  | RoAP3_B3_F<br>RoAP3_B3_R     |
|  | RoAP3_EuB3_F<br>RoAP3_EuB3_R |
| K-box region and MADS-box transcription factor family protein  | RoAG_C1_F<br>RoAG_C1_R       |
| K-box region and MADS-box transcription factor family protein  | RoAG_D1_F<br>RoAG_D1_R       |
|  | RoFD_F<br>RoFD_R             |
| 'EBP (phosphatidylethanolamine-binding protein) family protein | RoFT_DH_F<br>RoFT_DH_R2      |
| floral meristem identity control protein LEAFY (LFY)           | RoLFY_F<br>RoLFY_R           |
| AGAMOUS-like 20  | RoSOC1_F<br>RoSOC1_R         |
| ATC Centroradialis   | RoTFL1_F<br>RoTFL1_R         |
|  | RoUBC_F<br>RoUBC_R           |
|  | RoTCTP-S1<br>RoTCTP-AS1      |
|  | nptII_F6<br>nptII_R7         |
|  | GFP_F5<br>GFP_R6             |



**primers sequence for real time amplificatio**

CAGCTCGACCTTACTCTCG  
CTACAAGCTAGTGCCTTAGC  
GGGAGAAGGAGAAGGAGAAAGCAG  
AAGGCGGCAGGAGCGTATTAC  
TGGAGCGATATGAACAATAC  
GACCAGTTTCCCTGTGATTG  
CCACTTTTCACCATCGTTCACC  
CGTGGTTAAATCCTAGCTACC  
ATGATCTTAATGGTCTGAGCT  
AAGGTTGTGGTTGTTGTGG  
TCTGAGATTCGCTGAACTGC  
CGGTGAAGATTAGGATGATGA  
CACCACCAATCGTCAAGTCACC  
ACCTCAGCATCACAGAGCACAG  
CTCAATCATACGATAGGAG  
GGAGAGGTGTCTGGTTCTG  
AAAAGGGCGTTAGAAGAAAATGG  
GCCGATTCTCTGTTCTTGATC  
CACCATGCCTAGGGCTAGCGATCG  
CAACGCTAAACTCTCCTTCCA  
GGGAGCAAACACTACATCAATAAGCC  
CTCCTCAGCGCATTCTGAACC  
GCAAAAGACAACCAAGCCAAC  
TTCTTGCAGTTCGTCAATGC  
AAGCAGAAAGGTCTGGCAAACAGTG  
GCAGTTTCCCTCTGGGCATTGAAG  
GCCAGAGATTGCCCATATGTA  
TCACAGAGTCCTAGCAGCACA  
GAGGGAGCAACCAAGTTTCTG  
TGTAGTAGGCAAAGACCAAAGC  
GGGACTGGCTGCTATTGG  
CCCCTGATGCTCTTCGTC  
ACGTAAACGGCCACAAGTTC  
CTTGTAGTTGCCGTCGTCCT

**Table S3.** HSD-Tukey test for phenotypic data analyses of transformed and non-transformed (NT) plants for *Rosa Deltrimen* Guy Savoy®, *Rosa Deldog* Pimprenel the grey color indicates the RoFT events that are significantly different from the 1 plants.

FO: floral organs NbIN: number of internodes

Guy Savoy

Pimprenelle

**Date of flowering**

| Event | Letters |
|-------|---------|
| C3.1  | a       |
| C6.1  | a       |
| B1.2  | a       |
| C6.2  | a       |
| B1.1  | a       |
| C3.2  | a       |
| A2.2  | a       |
| A2.1  | a       |
| B1.3  | a       |
| NT    | c       |
| C5.1  | b       |

| Event   | Letters |
|---------|---------|
| 8.1_1.1 | a       |
| 8.1_1.2 | a       |
| 8.2_4.1 | a       |
| 8.1_2.1 | ab      |
| NT      | b       |

Guy Savoy

**Petal**

| Event | Letters |
|-------|---------|
| A2_1  | b       |
| A2_2  | ab      |
| B1_1  | ab      |
| B1_2  | ab      |
| B1_3  | ab      |
| C3_1  | ab      |
| C3_2  | ab      |
| C5_1  | ab      |
| C6_1  | a       |
| C6_2  | ab      |
| NT    | ab      |

**Stamen**

| Event | Letters |
|-------|---------|
| C3_1  | d       |
| C3_2  | bd      |
| B1_2  | bd      |
| B1_3  | bd      |
| B1_1  | abd     |
| C6_1  | abd     |
| C6_2  | abd     |
| C5_1  | abcd    |
| A2_2  | abc     |
| A2_1  | ac      |
| NT    | c       |

**Carpel**

| Event | Letters |
|-------|---------|
| C6_1  | a       |
| C6_2  | a       |
| B1_1  | a       |
| B1_2  | a       |
| B1_3  | a       |
| C3_1  | a       |
| C3_2  | a       |
| C5_1  | ab      |
| A2_2  | ab      |
| A2_1  | ab      |
| NT    | b       |

**Total**

| Event |
|-------|
| C3_2  |
| C6_1  |
| B1_2  |
| B1_3  |
| C3_1  |
| B1_1  |
| C6_2  |
| C5_1  |
| A2_2  |
| A2_1  |
| NT    |

Pimprenelle

**Petal**

| Event   | Letters |
|---------|---------|
| 8.2_2.1 | a       |
| 8.1_1.2 | a       |
| 8.2_4.1 | a       |
| 8.1_1.1 | a       |
| NT      | b       |

**Stamen**

| Event   | Letters |
|---------|---------|
| 8.2_2.1 | a       |
| 8.2_4.1 | ab      |
| 8.1_1.1 | a       |
| 8.1_1.2 | a       |
| NT      | b       |

**Carpel**

| Event   | Letters |
|---------|---------|
| 8.2_2.1 | a       |
| 8.2_4.1 | a       |
| 8.1_1.1 | a       |
| 8.1_1.2 | a       |
| NT      | a       |

**Total**

| Event   |
|---------|
| 8.2_2.1 |
| 8.2_4.1 |
| 8.1_1.1 |
| 8.1_1.2 |
| NT      |

Guy Savoy

**shootsize**

| Event | Letters |
|-------|---------|
| C3_1  | a       |

**NbIN**

| Event | Letters |
|-------|---------|
| B1_2  | a       |

**MeanSizeIN**

| Event | Letters |
|-------|---------|
| C3_1  | a       |

|      |     |
|------|-----|
| C3_2 | a   |
| A2_2 | ab  |
| B1_2 | ab  |
| B1_3 | ab  |
| C6_2 | ab  |
| C6_1 | ab  |
| B1_1 | abc |
| A2_1 | abc |
| C5_1 | bc  |
| NT   | c   |

|      |     |
|------|-----|
| C3_2 | a   |
| C6_1 | a   |
| C6_2 | a   |
| B1_1 | ab  |
| B1_3 | ab  |
| C3_1 | ab  |
| A2_2 | abc |
| A2_1 | bcd |
| C5_1 | cd  |
| NT   | d   |

|      |      |
|------|------|
| A2_2 | ab   |
| C5_1 | abc  |
| B1_3 | abcd |
| B1_2 | abcd |
| A2_1 | abcd |
| C3_2 | abcd |
| C6_2 | bcd  |
| NT   | bcd  |
| B1_1 | cd   |
| C6_1 | d    |

## Pimprenelle

### shootsize

| Event   | Letters |
|---------|---------|
| 8.1_1.1 | a       |
| 8.1_1.2 | a       |
| 8.1_2.1 | a       |
| 8.2_4.1 | a       |
| NT      | b       |

### NbIN

| Event   | Letters |
|---------|---------|
| 8.1_1.1 | a       |
| 8.1_1.2 | a       |
| 8.1_2.1 | ab      |
| 8.2_4.1 | b       |
| NT      | c       |

### MeanSizeIN

| Event   | Letters |
|---------|---------|
| 8.1_1.1 | a       |
| 8.1_1.2 | a       |
| 8.1_2.1 | a       |
| 8.2_4.1 | a       |
| NT      | a       |

## Guy Savoy

### PlantSize

| Event | Letters |
|-------|---------|
| A2.1  | a       |
| A2.2  | a       |
| B1.1  | a       |
| B1.2  | a       |
| B1.3  | a       |
| C3.1  | a       |
| C3.2  | a       |
| C5.1  | a       |
| C6.1  | a       |
| C6.2  | a       |
| NT    | a       |

## Pimprenelle

### PlantSize

| Event   | Letters |
|---------|---------|
| 8.1_1.1 | a       |
| 8.1_1.2 | a       |
| 8.1_2.1 | a       |
| 8.2_4.1 | a       |
| NT      | a       |

lle®.  
NT

**FO**

| Letters |
|---------|
| d       |
| bd      |
| bd      |
| bd      |
| bd      |
| abd     |
| abd     |
| abcd    |
| abc     |
| ac      |
| c       |

**FO**

| Letters |
|---------|
| a       |
| a       |
| a       |
| a       |
| a       |

**Table S4.** List of the papers on stable rose genetic transformatio

| <b>Reference</b>               | <b>Cultivar</b>                                  | <b>Ploidy</b> | <b>Modified character</b> |
|--------------------------------|--|---------------|---------------------------|
| Firoozabady et al. 1994        | 'Royalty'  | 4X            | Flower color              |
| Matthews et al. 1994           | <i>Rosa persica</i> X<br><i>xanthina</i>         | 4X            | Marker Gene               |
| Derks et al. 1995              | 'Sonia'  | 4X            | Disease resistance        |
| Souq et al. 1996               | ' <i>Deladel</i> '                               | 4X            | Plant Architecture        |
| Van der Salm et al. 1997, 1998 | 'Moneyway'                                       | 4X            | Root system               |
| Marchant et al. 1998           | ' <i>Glad tidings</i> '                          | 4X            | Disease resistance        |
| Dohm et al. 2001, 2002         | 'Heckenzauber' et 'Pariser charme'               | 4X            | Disease resistance        |
| Li et al. 2002                 | ' <i>Carefree</i> '                              | 4X            | Marker Gene               |
| Condliffe et al. 2003          | 'Only love'<br>'Romy' 'Fresco'<br>'Tineke' 'Glad | 4X            | Marker Gene               |
| Li et al. 2003                 | ' <i>Carefree</i> '                              | 4X            | Disease                   |
| Kim et al. 2004                | 'Tineke'   | 4X            | Marker Gene               |
| Chen et al. 2006               | <i>Rosa chinensis</i><br>Jacq.                   | ND            | Marker Gene               |
| Vergne et al., 2010            | <i>R chinensis</i><br>'Old Blush'                | 2X            | Marker Gene               |
| Zvi et al., 2012               | ' <i>Pariser</i> '                               | 4X            | Transcription             |
| Zakizadeh et al., 2013         | <i>Rosa hybrida</i><br>'Linda'                   | 4X            | Marker Gene               |
| Xing et al., 2014 a, b         | <i>R. rugosa</i>                                 | 2X            | Flowering                 |
| Randoux et al, 2014            | <i>Rosa hybrida</i>                              | 2x            | Flowering                 |
| Qiu et al, 2015                | <i>Rosa multiflora</i>                           | 2X            | Disease                   |
| Lee et al., 2020               | KR056002   | ND            | drought stress            |
| Liu et al., 2021               | <i>Rosa hybrida</i><br>'Samantha'                | ND            | Marker Gene               |

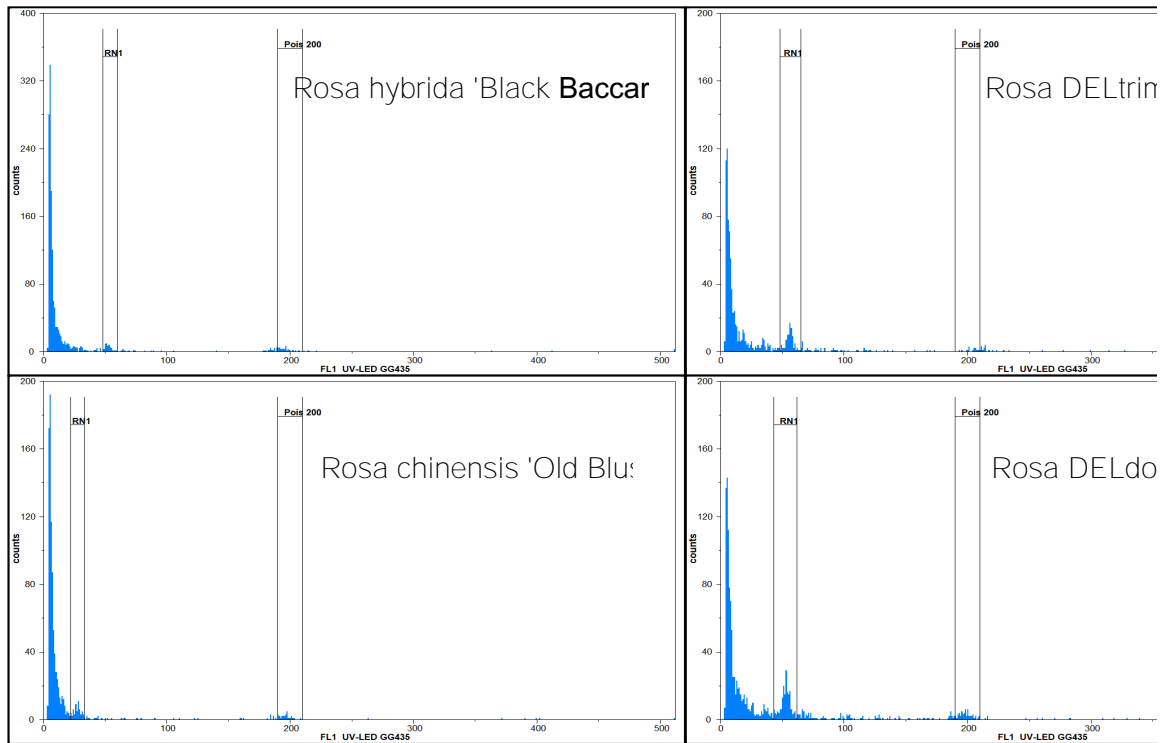
*ND: non determined*

n.

| <b>Transformation by</b> | <b>Integrated gene</b>                                  | <b>Target tissue</b> | <b>Efficiency of transformation</b> |
|--------------------------|---|----------------------|-------------------------------------|
| <i>A. tumefaciens</i>    | <i>Chalcone synthase</i>                                | Embryogenic callus   | ND                                  |
| <i>A. tumefaciens</i>    | $\beta$ - <i>glucuronidase</i>                          | Protoplastes         | ND                                  |
| <i>A. tumefaciens</i>    | $\beta$ - <i>glucuronidase (intron)</i> ,               | Embryogenic callus   | ND                                  |
| <i>A. tumefaciens</i>    | <i>Chalcone synthase</i>                                | Embryogenic callus   | 1 to 2 %                            |
| <i>A. rhizogenes</i>     | <i>Rol gene</i>   | Embryogenic callus   | ND                                  |
| biolistic                | $\beta$ - <i>glucuronidase</i>                          | Embryogenic callus   | ND                                  |
| <i>A. tumefaciens</i>    | <i>Chitinase</i><br><i>Glucanase</i><br><i>Lysozyme</i> | Somatic Embryo       | ND                                  |
| <i>A. tumefaciens</i>    | $\beta$ -   | Somatic Embryo       | ND                                  |
| <i>A. tumefaciens</i>    | $\beta$ - <i>glucuronidase (intron)</i>                 | Somatic Embryo       | ND                                  |
| <i>A. tumefaciens</i>    | <i>Ace-AMP1</i>   | Embryogenic          | 9%                                  |
| <i>A. tumefaciens</i>    | <i>GFP</i>  | Embryogenic          | 6,6%                                |
| <i>A. tumefaciens</i>    | $\beta$ - <i>glucuronidase</i>                          | organogenic callus   | ND                                  |
| <i>A. tumefaciens</i>    | $\beta$ - <i>glucuronidase</i>                          | Embryogenic callus   | 3-9%                                |
| <i>A. tumefaciens</i>    | <i>PAP1</i>   | Embryogenic          | ND                                  |
| <i>A. tumefaciens</i>    | <i>PSAG12-ipt</i>                                       | Embryogenic callus   | 0.1                                 |
| <i>A. tumefaciens</i>    | $\beta$ - <i>glucuronidase FT Prunus</i>                | Embryogenic callus   | 0.00114                             |
| <i>A. tumefaciens</i>    | <i>RoKSN</i>  | Embryogenic          | 0.17-12%                            |
| <i>A. tumefaciens</i>    | <i>MLO genes</i>  | Embryogenic          | ND                                  |
| <i>A. tumefaciens</i>    | <i>SOD2</i>   | Embryogenic          | ND                                  |
| <i>A. tumefaciens</i>    | <i>GFP</i>  | Embryogenic callus   | 6%                                  |

**Figure S1** : Histogram (A) and table (B) of fluorescent intensity on flow cytomet tetraploid genotype (*Rosa hybrida* 'Black Baccara'®), diploid genotype (*Rosa ch.* the two genotypes to be tested (*Rosa DELtrimen* 'Guy Savoy'® and *Rosa DELdog*

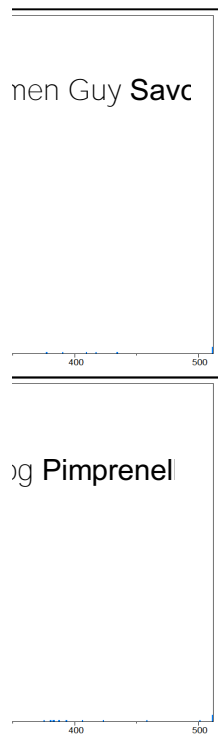
**A**



**B**

| genotype                           | ploidy expected | Pea   |        |        |
|------------------------------------|-----------------|-------|--------|--------|
|                                    |                 | Count | %Gated | Mean-x |
| <i>Rosa hybrida</i> 'Black Bacc    | 4x              | 36    | 2.5    | 196.53 |
| <i>Rosa chinensis</i> 'Old Blush   | 2x              | 25    | 2.67   | 197.2  |
| <i>Rosa DELtrimen</i> 'Guy Savoy'® | ?               | 16    | 1.81   | 203.44 |
| <i>Rosa DELdog</i> 'Pimprenelle'®  | ?               | 52    | 4.1    | 198.92 |

try of the controls of  
*inensis* 'Old Blush'),  
 g 'Pimprenelle'®) and



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| CV-x% | Rosa  |        |              |       | ploidy defined |
|-------|-------|--------|--------------|-------|----------------|
|       | Count | %Gated | Mean-x       | CV-x% |                |
| 2.16  | 52    | 3.61   | <b>52.64</b> | 4.54  | 4x             |
| 2.03  | 54    | 5.78   | <b>27.85</b> | 9.24  | 2x             |
| 2.4   | 91    | 10.27  | <b>56.2</b>  | 5.86  | 4x             |
| 2.38  | 172   | 13.56  | <b>53.08</b> | 6.73  | 4x             |