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Natural variation suggests new mechanisms for bract development in *Arabidopsis*, desynchronising bract suppression from the floral transition

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1 SUMMARY

- 2 ● Bracts, the leaves subtending flowers, were lost multiple times in angiosperms, including
3 in *Brassicaceae*, where their development is blocked early. *Arabidopsis* mutants that
4 restore bract formation suggest that flower identity genes suppress the vegetative
5 program of bract development, but the exact mechanisms and the evolutionary origin of
6 bract loss remain unclear.
- 7 ● We exploited natural variation in bracts that form only at the base of flowering branches
8 in *Arabidopsis*, to study bract development and its connection to floral transition. We
9 combined multiscale phenotyping, quantitative genetics, meristem imaging, time-series
10 transcriptomics and curve registration to capture the genetic and developmental
11 mechanisms unlocking bract development during floral transition.
- 12 ● We mapped four Quantitative Trait Loci controlling bracts, excluding known bract mutant
13 genes. We demonstrated *LEAFY* and other flower regulators were not involved and
14 identified lists of new candidate genes and pathways, such as the anthocyanin pathway.
15 We found that bract develops when gene expression is desynchronised from the floral
16 transition, either later or earlier, revealing a more complex landscape than the previously
17 proposed prolonged vegetative state.
- 18 ● We identified new mechanisms unlocking bract development. This natural variation
19 sheds a new light on development canalisation during floral transition and on bract loss
20 evolution.

21 5–8 key words

22 *Arabidopsis* natural variation, bract, evolutionary loss, floral transition, heterochrony,
23 quantitative genetics, time registration, time-series transcriptomics.

24 **Introduction**

25 Morphological evolution sometimes proceeds by losing structures or by regaining lost traits.
26 The genetic and developmental mechanisms leading to a trait loss or regain are being
27 elucidated (Cronk 2009; Sadier et al. 2022). Trait loss can simply result from gene(s) loss (Xu et
28 al. 2019). However, pleiotropy (one gene having different functions) can limit gene loss if it
29 causes detrimental effects and reduces fitness (Helsen et al. 2020). Also, a full genetic knock-
30 out of a trait may hinder its regain (Sadier et al. 2022). Hence, more studies are needed to
31 unravel the mechanisms behind the evolution of trait loss or regain.

32 In flowering plants, bracts provide an interesting model to address this loss and regain question
33 as they have been repeatedly lost and sometimes regained in different groups. Bracts refer to
34 any leaves developing in the inflorescence of angiosperms such as the edible leaves of an
35 artichoke. More accurately in botany, bracts are the leaves subtending a single flower, at the
36 junction of the floral peduncle with the stem (Dinneny et al. 2004; Endress 2006; Prenner et al.
37 2009). Bracts have evolved a wide diversity aligned to various adaptive functions:
38 photosynthesis, mechanical protection, pollinator attraction or seed dispersion. Some of these
39 adaptive changes have resulted in bract reduction or loss, like in *Poaceae* (grasses) or
40 *Brassicaceae* (cabbages). This loss may be an evolutionary adaptation to specific pollinators or
41 anemogamy, privileged resource allocation into reproductive versus vegetative organs or
42 developmental constraints (Whipple et al. 2010). To investigate the underlying genetic and
43 developmental mechanisms, several studies have been led in *Poaceae* (using rice, maize, wheat
44 and barley) and in *Arabidopsis thaliana* for *Brassicaceae*.

45 In these two groups, the reduced bracts are not strictly homologous. In *Poaceae* inflorescences
46 (ear, panicles or tassels), bracts are suppressed at major branching points while the bracts
47 closely associated with the floral unit (florets) are maintained (Whipple 2017; Xiao et al. 2022).
48 In *Brassicaceae*, the suppressed bract directly subtends the flower. Despite these differences,
49 two common points are shared. First, bract reduction is not total. Morphological deformations
50 (Kwiatkowska 2006) and localised gene expressions, such as *A. thaliana* *FILAMENTOUS FLOWER*
51 (*FIL*) or its maize ortholog *Zea mays yabby15* (*Zyb15*), indicate that a bract domain forms but
52 fails to outgrow (Long and Barton 2000; Dinneny et al. 2004; Whipple et al. 2010). In *A.*

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53 *thaliana*, this domain is incorporated in floral tissues, remaining cryptic (Heisler et al. 2005;
54 Goldshmidt et al. 2008). Second, in both families, mutants can restore a bract from the cryptic
55 domain. This suggests that a gene regulatory network (GRN) actively suppresses bract
56 outgrowth in this domain. In *Poaceae* model plants, bract regain in mutants is often interpreted
57 as a heterochrony, or a change in developmental timing (Alberch et al. 1979). Bracts are
58 compared to leaves produced during juvenile phases, so the persistence of this developmental
59 program in the inflorescence suggests a ‘prolonged vegetative phase’ (Itoh et al. 1998;
60 Kawakatsu et al. 2006; Chuck et al. 2007; Kawakatsu et al. 2009; Chuck et al. 2010; Wang et al.
61 2021). This hypothesis, however, does not offer clear insights into the precise mechanisms
62 controlling bract regain in mutants, nor into the evolutionary scenario of bract loss in *Poaceae*.
63 Studies on bract mutants in *Poaceae* and *A. thaliana* have identified two GRNs controlling bract
64 suppression. In *Poaceae*, the GRN is organised around *NECK LEAF1* / *TASSEL SHEATH1* / *THIRD*
65 *OUTER GLUME1* (*NL1*/*ISH1*/*TRD1*, or *NTT*) in rice, maize and barley respectively (Wang et al.
66 2009; Whipple et al. 2010; Houston et al. 2012). Loss-of-function mutants in grass species
67 produce bracts (Whipple et al. 2010; Wang et al. 2021), but not in *A. thaliana* mutants of the
68 orthologous gene *HANABA TARANU* (*HAN*) (Zhao et al. 2004). Upstream, *NTT* is directly
69 regulated by *SQUAMOSA PROMOTER BINDING PROTEIN LIKE* (*SPL*) transcriptional regulators
70 (Wang et al. 2009; Chuck et al. 2010; Chuck et al. 2014), themselves targeted by
71 microRNA156/529 (miR156/529) (Chuck et al. 2007; Wang et al. 2021). The miR156/*SPL* balance
72 regulates phase transitions from vegetative to reproductive stages in angiosperms (Wu and
73 Poethig 2006; Wang and Wang 2015), providing a genetic module controlling heterochrony in
74 plants (Buendía-Monreal and Gillmor 2018). This supports the interpretation that bract regain
75 in mutants is a heterochronic phenotype. Downstream, *NTT* influences genes involved in
76 hormone signalling, meristem and boundary identities, or leaf development (Xiao et al. 2022).
77 Yet, few direct *NTT*-target genes have been identified, such as the *PLASTOCHRON1* (*PLA1*) and
78 *PLA2* genes in rice (Wang et al. 2021). These genes encode two enzymes whose function has
79 not been clearly identified (Miyoshi et al. 2004; Kawakatsu et al. 2006). Hence, *NTT*-
80 downstream mechanisms of bract repression need clarification.

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81 In *A. thaliana*, bract mutants revealed different genes from the *Poaceae* orthologs, all
82 connected to the floral meristem identity (FMI) pathway. This genetic program specifies a floral
83 rather than a shoot identity (Weigel 1995). Impairing FMI leads to flower-to-shoot conversions
84 by restoring vegetative hallmarks into flowers: perturbed floral organ identity and phyllotaxis,
85 internode elongation, indeterminacy and also, bract outgrowth. In *A. thaliana*, FMI relies on a
86 complex gene regulatory network orchestrated by the transcription factor *LEAFY* (*LFY*)
87 (Siriwardana and Lamb 2012): *lfy* mutants fail to produce proper flowers and show diverse
88 bract outgrowths (Schultz and Haughn 1991; Weigel et al. 1992). Other FMI-related
89 transcription factors also cause bract de-repression when mutated alone or in combination,
90 suggesting they also act as bract-repressors: *UNUSUAL FLORAL ORGANS* (*UFO*) (Levin and
91 Meyerowitz 1995; Hepworth et al. 2006), *APETALA1* (*AP1*) (Irish and Sussex 1990; Bowman et al.
92 1993), *BLADE ON PETIOLE1* (*BOP1*) and *BOP2* (Hepworth et al. 2005; Norberg et al. 2005; Xu et
93 al. 2010; Chahtane et al. 2018), *PUCHI* (Karim et al. 2009), *LATE MERISTEM IDENTITY 1* (*LM1*),
94 *AGAMOUS-LIKE 24* (*AGL24*), *SHORT VEGETATIVE PHASE* (*SVP*) (Xu et al. 2010; Grandi et al. 2012),
95 *SUPPRESSOR OF CONSTANS 1* (*SOC1*), *SEPALLATA3* (*SEP3*) (Liu et al. 2009), *FRUITFUL* (*FUL*)
96 (Melzer et al. 2008; Balanzà et al. 2014), *TERMINAL FLOWER 1* (*TFL1*) (Shannon and Meeks-
97 Wagner 1991; Penin 2008) and *FILAMENTOUS FLOWER* (*FIL*) (Levin and Meyerowitz 1995; Sawa
98 et al. 1999; Siegfried et al. 1999). Fewer genes have been identified as promoting bract
99 outgrowth, such as *JAGGED* (Dinneny et al. 2004; Ohno et al. 2004), *AINTEGUMENTA* (*ANT*) and
100 *AINTEGUMENTA-LIKE6* (*AIL6*) (Manuela and Xu 2024 Mar 28). In all these studies, bract de-
101 repression is accompanied by pleiotropic phenotypes affecting flowers, branching or flowering
102 time. Bracts, together with chimeric shoot-flowers, can also be induced in wild-type *A. thaliana*
103 under specific light treatments (Hempel and Feldman 1995; Hempel et al. 1998). Finally, the
104 genetic ablation of flowers induces bract outgrowths (Nilsson et al. 1998). Altogether, this
105 suggests that FMI establishment is responsible for bract repression, although the precise
106 mechanisms remain unclear.

107 To summarise, thanks to bract mutant studies, current models propose different GRNs to
108 acquire the ability to suppress bract, either from boundaries in *Poaceae* or from flowers in
109 *Brassicaceae*. In this latter case, it is unclear how FMI genes mostly expressed in flowers act

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110 non-cell autonomously on the bract domain. Also, how this network acquired this new function
111 remains to be determined.
112 This study revisits bract inhibition in *A. thaliana* with natural variation. Important differences
113 suggest that the bracts naturally forming at the base of inflorescences involve developmental
114 mechanisms distinct from mutant bracts and that may be more similar to bract-bearing species.
115 Using two accessions, we mapped this heritable trait to four Quantitative Trait Loci (QTL).
116 Transcriptomic profiling of bract-producing meristems indicates that during floral transition, the
117 stage at which bract develops, the meristematic transcriptomes of the two accessions diverge
118 the most, with numerous desynchronisation of gene expression relative to the flowering event.
119 Challenging the model that FMI genes inhibit bracts, our results question whether changes in
120 the timing of gene expression may play a role in canalising phenotypes during floral transition
121 and in the evolution of bract loss.

122

123 **Materials and Methods**

124 **Plant growth conditions**

125 Seeds were sown on peaty-clay soil, stratified at 4°C for at least two days, and watered with
126 fertiliser (18-10-18 N-P-K) under LED lighting (sunlight spectrum NS12, 150 $\mu\text{mol.m}^{-2}\text{s}^{-1}$).
127 Three different day/night regimes were used in the experiments: short-days (SD) with 8h light
128 and 16h dark; long-days (LD) with 16h light and 8h dark and continuous light (CL) with 24h light.
129 Temperature and humidity are controlled as follows: 22°C and 60% humidity during light for LD
130 and CL conditions, and 18°C and 70% humidity constantly in SD and during night time in LD. For
131 the Bulk Segregant Analysis and the RNAseq time course, plants were grown 20 days in SD
132 before switching to LD. For the RIL phenotyping assays, plants have been directly cultured in LD.

133 **Plant materials**

134 *A. thaliana* natural accessions and associated RILs were obtained from the Versailles
135 *Arabidopsis* Stock Centre (VASC), *Col-0*: 186AV, *Tsu-0*: 91AV, RIL set name: 3RV. F1 and F2
136 plants used for the BSA were generated by crossing the parents *Tsu-0* and *Col-0* in both
137 directions. The following strains were obtained from the NASC and are in *Col-0* background if
138 not otherwise mentioned: *ufo-1*: N16361 (*Col-2* background); *jag-5d*: N9506 (background: *Col-0*
139 *gl1 pop1*); *tfl1-13*: N6237; *tfl1-14*: N6238. *puchi-1*, *bop1-4 x bop2-11* and *puchi-1 x bop1-4 x*

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140 *bop2-11* (Karim et al. 2009), *lfy-12* (Maizel and Weigel 2004), *soc1-2* (Michaels et al. 2003) and
141 *svp x agl24 x soc1* (Liu et al. 2009) were described previously. Plants expressing
142 pLFY::2xmCherry-N7 ; 35S::Lti6b-YFP (in *Col-0* and *Tsu-0*) were generated for this study.
143 *Tarenaya hassleriana* and *Gynandropsis gynandra* seeds were kindly provided by Pr Eric Schranz
144 and Frank Becker, from Wageningen University (WUR, Holland). *Lunaria annua* and *Allaria*
145 *petiolata* plants are spontaneous specimens found outside the laboratory, in France. The
146 phylogeny of *Brassicaceae* tribes used in Fig. S1 is extracted from the Brassibase website
147 (Kiefer et al. 2014).

148 **Plasmid constructions and plant transformation**

149 pLFY::2xmCherry-N7: since no polymorphism was sequenced in the *LFY* promoter between *Col-*
150 *0* and *Tsu-0* accessions, a sequence starting at -2277pb upstream of the ATG was amplified by
151 PCR from *Col-0* genomic DNA using
152 5'GGGGACAACCTTTGTATAGAAAAGTTGATCCATTTTTTCGCAAAGG and
153 5'GGGGACTGCTTTTTTGTACAAACTGAATCTATTTTTCTCTCTCTCTATC primers. PCR fragments
154 were purified and inserted into pDONR P4-P1R with a gateway BP reaction. This plasmid was
155 then recombined in a three-fragment gateway reaction with 2xmCherry pDONR211 and N7-tag
156 pDONR P2R-P3 (containing the nuclear tag N7 and a stop codon) and the destination vector
157 pK7m34GW. 35S::Lti6b-YFP: a pDONR P2R-P3 plasmid containing the sequence of the plasma-
158 membrane protein Lti6b (Cutler et al. 2000) was recombined in a three-fragment gateway
159 reaction with a 35S pDONR P4-P1R and the YFP pDONR P2R-P3 into the destination vector
160 pB7m34GW. Both constructs were then transformed into *Agrobacterium tumefaciens*
161 C58pMP90 strain by electroporation and transformed into both *Col-0* and *Tsu-0* plants by floral
162 dipping (Clough and Bent 1998). For each construct-by-genotype combination, several
163 independent transgenic lines were selected in T2 for a single insertion event based on 3:1
164 resistant:sensitive mendelian segregation of the resistance provided by the transgene. The
165 expression patterns of pLFY were reproducible between selected lines and matched published
166 data for *Col-0*. We were unable to get a 35S::Lti6b-YFP line with a membrane signal as strong in
167 *Tsu-0* as in *Col-0* (Fig. 2 and S2). However, despite the weak YFP signal, the morphology of the
168 tissue could still be captured.

169 **Microscopic meristem imaging and image analysis**

170 Meristems were imaged using a Scanning Electron Microscope (Hirox 3000 SEM), or with a
171 confocal microscope Zeiss 700 LSM, according to the manufacturer's instruction and without
172 prior fixation. Multitrack sequential acquisitions were performed as follows: YFP, excitation
173 wavelength (ex): 488 nm, emission wavelength (em): 300–590 nm; mCherry, ex: 555 nm, em:
174 300–620 nm. Detection wavelengths and laser power were identical for *Col-0* and *Tsu-0*, PMT
175 voltage was identical for mCherry to allow comparisons of pLFY signal intensity in the two
176 genotypes. The YFP PMT voltage was optimised on each plant. Confocal images were processed
177 using imageJ (Schneider et al. 2012): maximum projections of mCherry channel and 16-bit-
178 transformed standard deviation projections of the YFP channel were merged in a composite
179 image. mCherry intensities were unchanged while brightness and contrast of the YFP channel
180 were optimised on each plant to provide the best morphological outlines of the tissues.

181 **Macroscopic plant phenotyping**

182 Macroscopic pictures were taken using different devices, according to the manufacturer's
183 instructions: Keyence VHX-900F, Canon EOS 450D camera, camera device of a Samsung Galaxy
184 A10 and a Ulefone Armor X5 pro.

185 **Phenotypic measurements**

186 Basal bract score was determined by counting all bracts in the main stem and cauline branches,
187 normalising by the total number of branches (excluding rosette branches, **Fig. 1E**); the
188 inspection was limited to the first five flowers, especially for mutants. The number of bracts
189 was counted after internodes elongation on the last upper cauline branch to ensure bracts
190 were visible to the naked eye. Flowering time was measured with different methods
191 (mentioned in the main text): the number of days from the start of transfer to in growth
192 chambers to the day of bolting, the day when the first flower blooms (open petals), or as the
193 cumulated number of leaves (rosette only or rosette and cauline). For plastochron
194 measurements, several plants of both genotypes were synchronised and grown in the same
195 condition. Each sampling day after transfer to a long day, 5 to 10 plants were randomly
196 dissected under a binocular dissecting scope, removing and counting all organs (leaves or
197 flowers) from the first leaf to the youngest organ visible on the main meristem. The youngest

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198 organs were counted as soon as they were separated by a boundary (corresponding to stage-2
199 flowers as defined by (Smyth et al. 1990).

200 **DNA extraction and sequencing for Bulk Segregant Analysis (BSA)**

201 For the BSA, an F2 mapping population of 684 plants was generated by crossing (*Col-0* x *Tsu-0*)
202 in both directions. The bulk segregant analysis was split into four replicates. A 1cm² leaf sample
203 was sampled for each F2 plant, kept at -20°C and genomic DNA was extracted individually if the
204 plant was selected in one of the bulk. Genomic DNA of 56 and 17 plants were selected and
205 pooled in the bulks of low and high bract-score plants, respectively. The genome of the parental
206 lines was re-sequenced using genomic DNA from bulk *Tsu-0* and *Col-0* seedlings, respectively.
207 All genomic DNAs were extracted and purified using a CTAB-based protocol (cetyl
208 trimethylammonium bromide), following instructions as in (Healey et al. 2014).

209 Each DNA bulk was prepared by pooling the genomic DNA of selected plants in equal quantities,
210 to reach a final concentration between 13 and 25 ng/μl. Pooled DNA bulks and parental DNA
211 were used to prepare libraries and sequenced on BGISEQ-500WGS according to the
212 manufacturer's instructions, yielding 5 Gb data of 100bp paired-end reads per library (target
213 coverage of 40X).

214 **DNA sequencing analysis and genomic variant analysis**

215 Sequencing data consists of two parental plus two bulks of sequencing data. A genomic variant
216 analysis was performed on each dataset following the workflow of short variant discovery
217 previously described in (Besnard et al. 2020). This resulted in four gVCF files (one per sample)
218 generated by the HaplotypeCaller tool of GATK (v3.8, McKenna et al., 2010). TAIR10 was used
219 as the *A. thaliana* reference genome. Then, the two parental gVCF were first joint-genotyped
220 using GATK's tool GenotypeGVCFs to emit a common vcf file for the two samples. This file was
221 used to select a list of specific SNPs and small indels of *Tsu-0* (91AV stock) versus *Col-0* (186AV
222 stock), filtering for positions with coverage metric DP>10. This reference list of *Tsu-0*
223 polymorphisms was then used as the --dbsnp option in a second pass of joint genotyping using
224 all four gVCF as input (two parental samples plus the two bulks) to emit a common vcf file.
225 Finally, relevant polymorphic positions from the reference list in the two bulks were selected
226 after filtering for a depth ≥3.

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227 **QTL mapping from Bulk Segregant Analysis**

228 QTL identification was carried out using the QTLseqr package (Mansfeld and Grumet 2018)
229 according to user instructions. After filtering data with the following parameters (refAlleleFreq
230 = 0, minTotalDepth= 10, maxTotalDepth = 90, minSampleDepth = 10, minGQ= 99,
231 depthDifference = 15), the deltaSNP index (Takagi et al. 2013) was generated and loci that
232 reached the ~95\% confidence interval were retained.

233 **QTL mapping using Recombinant Inbred Lines**

234 Genotyped RILs from *Col-0* x *Tsu-0* (3RV) are publicly available in the VASC. Based on BSA
235 results, a panel of 55 RIL were selected from their known genotypes on chromosomes 1 and 5
236 using GGT 2.0 software (van Berloo 2008). The detailed genotypes of each line used in this
237 study are available in **Table S6**. The presence of basal bracts was quantified in each line using
238 the bract score. QTL mapping was performed with R/qtl software according to user instructions
239 (Broman et al. 2019): we used the scanone function with the mean value of the bract score for
240 the 55 tested RILs as a trait and significant thresholds were computed by setting a permutation
241 number to 2000. For the bract score, the “non-parametric” (np) statistical model was used
242 while for paraclade number, we used a normal model implemented with the hk method. To
243 look for QTL interactions, the scantwo function was used with the normal model and the hk
244 method since the np model is not implemented for this function. MQM was performed with
245 default parameters. The (broad-sense) heritability for the bract score was defined as $H^2 = (var_T -$
246 $var_E) / var_T$, with var_T and var_E being the total and environmental variance, respectively. var_T
247 was computed as the total variance of the bract score of each plant over the entire QTL
248 mapping dataset (1830 RIL plants and 780 control parents split over 12 experiments) while var_E
249 was computed as the pooled variance of the line variances (computed over all plants of a line
250 across experiments). Confidence intervals of H^2 were provided by bootstrapping 70% of the
251 strain 1000 times.

252 **KASP genotyping**

253 We selected 19 new genotyping markers from the 140SNPvCol marker set (Lutz U. et al. 2017)
254 to cover the two large QTLs mapped in chr1, 1a and 1b (**Fig. S5B**) and corresponding
255 kompetitive allele-specific PCR (KASP) oligos were ordered to Biosearch Technologies™ LGC Ltd.

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256 To further reduce the intervals, we designed two new SNP markers targeting *Tsu-0*
257 polymorphisms identified in our whole-genome re-sequencing data. Specific KASP oligos (three
258 per SNP) were designed by LGC from the 100-bp sequence surrounding the SNP. All 21 new
259 markers (**Table S7**) were validated on parental genomic DNA (*Col-0* and *Tsu-0*) in a KASP assay
260 before their use with recombinant inbred lines. Clean genomic DNA from CTAB-based
261 extraction was used for all samples. For each marker, 2.5 μ L of DNA (diluted to a final
262 concentration of 5 ng/ μ L), 2.5 μ L of KASP-TF V4.0 2X Master Mix low ROX and 0.07 μ L KASP
263 assay mix were mixed in 384-well plates and the genotyping assays were run in a QuantStudio 6
264 Flex (Applied Biosystems), using standard user guidelines for thermal cycling and final
265 fluorescence analysis.

266 **Fine mapping using Heterologous inbred families**

267 Using F6 genotyping data for the 3RV RIL set from the VASC, we selected 3 RILs heterozygous in
268 a region overlapping QTL1a, QTL1b and the interval in-between: RIL 334, 488 and 478,
269 respectively (**Fig. 3** and **Fig. S5**). For RIL 334 and 488, we genotyped by KASP 160 segregating F8
270 progenies and selected 5 and 8 plants, respectively, with homozygous recombination inside the
271 QTL region under study. For RIL 478, we genotyped 20 plants and selected only one; genotyping
272 reactions were performed using PCR primers designed to amplify the published marker of the
273 VASC (Simon et al. 2008)(**Table S8**) and the results were read by Sanger sequencing. All selected
274 F8 plants were selfed to generate an inbred line of a fixed genotyped (F9), the collection of F9
275 inbred lines forming a Heterologous Inbred Family (HIF). HIF lines (with at least 20 plants per
276 line) were phenotyped for the bract score with parental control in the same experiment.

277 **Biological sample preparation for the transcriptome time-series profiling**

278 After 20 days in SD, *Tsu-0* and *Col-0* meristems were dissected every day in LD conditions, in
279 order to capture the precise developmental stages (especially stages T and F, see below). The
280 mutants *lfy-12*; *puchi1 x bop1 x bop2*; and *jag5-D* meristems were dissected every day starting
281 from 1 week after LD transfer to target the stage F. Three independent biological experiments
282 were performed with 5 to 11 meristems per replicate. Stages are defined as follows: V, the day
283 when plants were transferred to LD from SD; L, after 4 to 5 days of LD (identical for both *Col-0*
284 and *Tsu-0*, which have the same meristem shape at this time); T, the main meristem enlarges

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285 and domes, (Kwiatkowska 2008; Kinoshita et al. 2020), and lateral meristems starts being visible
286 at the axils of young leaf primordia; F, all young organs in the meristems were identified as
287 flowers (note that this stage occurred several days before bolting). Stage F in mutants was
288 defined when several rounded primordia become visible at the SAM (that will become branch-
289 like flowers or only branches). Dissections were performed from 9:00 a.m. to noon, by
290 alternating between *Tsu-0* and *Col-0*. Micro-dissected meristems were snap frozen in liquid
291 nitrogen and stored at -80°C until RNA extraction. To avoid the induction of stress-related gene
292 expression, meristem dissection did not exceed 3 min between the first organ removal and
293 freezing. For each replicate, pooled meristems were ground with a Rockyll tissue lyser
294 according to the manufacturer's instructions. RNA was extracted using the PicoPure RNA
295 Isolation Kit (Arcturus, Catalog KIT0202) according to the standard protocol. RNA concentration
296 ranged from 3 to 64 ng/μl (average 23ng/μl), with a RIN value between 5.6 and 7.6 (average
297 6.8).

298 **RNA-sequencing**

299 Library preparation was made using NEBNext® Ultra™ II Directional RNA Library Prep Kit for
300 Illumina (New England Biolabs); NEBNext® Poly(A) mRNA Magnetic Isolation Module (New
301 England Biolabs); and NEBNext® Multiplex Oligos for Illumina (Index Primers Sets 1, 2 et 3),
302 from 40ng of RNA and sequenced using the NextSeq500 (Illumina). Sequence quality was
303 controlled using the Sequencing Analysis Viewer, sequences that did not pass the quality filter
304 were removed. Following QC an average of 43 million sequences per sample was achieved.

305 **RNA-seq analysis (pre-processing procedures)**

306 Quality filtering using Trimmomatic (Bolger et al. 2014) retained for all samples 96% of the
307 reads, which were aligned to TAIR10 genome using STAR (Dobin et al. 2013) with the following
308 parameters: non canonical splice junctions were removed, multi-mapping reads were limited to
309 10, and only reads mapped once were considered to determine splicing junctions. Between 88
310 and 97% of the reads were mapped to a simple locus. Normalisation of read counting was
311 performed using the R Bioconductor packages DESeq (Love et al. 2014), with the following
312 parameters: genes for which the total number of reads was below 10 were discarded, and data
313 were transformed with Variant Stabilising Transformation (VST) function (Anders and Huber

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314 2010), to harmonise the variance. Consistency between biological replicates was verified using
315 a Principal Component Analysis on all samples with VST-transformed data.

316 **RNA-seq analysis: Differential Gene Expression (DGE) analysis**

317 Differential analysis was performed using the R Bioconductor package edgeR (McCarthy et al.
318 2012). Reads were first normalised using TMM (Trimmed mean of M-values) to reduce library-
319 specific biases. Normalisation factors were between 0.94 and 1.049. A generalised linear model
320 was applied for the analysis of differentially expressed genes (DEG). Three types of DEG analysis
321 were considered: DEG at each stage between the different genotypes, DEG between the stage
322 within the same genotype, and DEG across all conditions (stages and genotypes). Multiple DEG
323 analyses were corrected using Benjamin-Hochberg correction, and genes with a p-value < 0.05
324 were retained.

325 **RNA-seq analysis: clustering gene expressions correlating with bract presence**

326 To identify candidate genes that may act as positive or negative bract regulators (see **Figure**
327 **5G**), we first calculated the minimum and maximum expression levels among stages with and
328 without bracts. If the minimum expression level in the stages with bracts was greater than or
329 equal to the maximum expression level in the stages without bracts, the genes were considered
330 positive bract regulators. If the maximum expression level in the stages with bracts was lower
331 than or equal to the minimum expression level in the stages without bracts, the genes were
332 considered positive bract regulators. If neither of these conditions were satisfied, genes were
333 discarded.

334 **Temporal registration of gene expression dynamics**

335 To align gene expression profiles of *A. thaliana* *Tsu-0* (query data) with *Col-0* (reference data),
336 we utilised the curve registration method in the R package greatR (Kristianingsih 2024). This
337 approach involved shifting the gene expression profiles of *Tsu-0* across developmental stages
338 (V, L, T, F) using shift factors ranging from -1 to 1; a stretch factor was not applied due to the
339 two datasets being over similar times. The optimal shift parameter for each gene pair was
340 identified by maximising the log-likelihood. The fit with the best registration factors for each
341 gene was then compared to the fit with a non-registered model (without transformation) using
342 the Bayesian Information Criterion (BIC) statistic. A lower BIC score for the registered model

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343 (with transformation) indicated that the gene expression dynamics of *Tsu-0* and *Col-0* were
344 similar, and the expression profile differences could be resolved through registration.
345 Conversely, a higher BIC score suggested that the profiles were best described by two individual
346 curves, i.e. not aligned. Prior to transformation, gene expression levels in both datasets were
347 centred and scaled using the z-score scaling method. The standard deviation value for the
348 replicates at each time point was set to 0.01.

349 **GO terms analysis**

350 GO term enrichment analyses were performed using either the 'Single Enrichment Analysis'
351 tool from AgriGOv2 (Tian et al. 2017) with default parameters (Fisher's test with Yekutieli
352 adjustment method) or clusterProfiler 4.0 (Wu et al. 2021) with default parameters (enrichGO
353 and simplify functions with "BH" adjustment method). The Rich factor was computed by
354 dividing the number of genes associated with a GOterm ("Count") by the numerator of the
355 "BgRatio" computed by the enrichGO function.

356 **Gene selection inside genetic mapping intervals**

357 The R library 'GenomicFeatures' (Lawrence et al. 2013) was used to import gene information
358 from the most recent annotation file of *A. thaliana* at the gff format (Cheng et al. 2017). Custom
359 R scripts were used to intersect all gene loci falling within genetic mapping data and provide for
360 each gene information from RNA-seq analysis and genomic variant analysis (see Table S1 and
361 Table S2). We used snpEff v5.0d (Cingolani et al. 2012) and its putative functional categories
362 (HIGH/MODERATE/LOW/MODIFIER) to predict the functional impacts of the genomic variants
363 over regions covering each annotated gene of the interval, including promoter (2 kbp upstream
364 of the transcriptional start site) as well as 500-bp downstream region in the case of miRNA. In
365 Table S1 and S2, additional gene information was retrieved from ThaleMine (Pasha et al. 2020)
366 and GO terms from the TAIR bulk data retrieval tool.

367 **Data handling, data visualization and descriptive statistics**

368 The R software was used (R Core Team 2018), especially the following packages: Bioconductor
369 packages (Huber et al. 2015) for the analysis of omics data, especially GenomicFeatures, IRange
370 (Lawrence et al. 2013), rtracklayer (Lawrence et al. 2009), clusterProfiler (Wu et al. 2021),
371 limma (Ritchie et al. 2015), DESseq2 (Love et al. 2014), edgeR (Chen et al. 2016) and

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372 org.At.tair.db (Carlson 2024); dplyr (Wickham et al. 2023), tidyr (Wickham et al. 2024) and
373 reshape2 (Wickham 2007) for data manipulation and ggplot2 for almost all plots (Wickham
374 2016), with viridis and plasma (Garnier et al. 2024) for some colour optimization.

375 **Text and english**

376 Language correction software with artificial intelligence was occasionally used throughout the
377 text to ensure correct spelling and grammar as well as the most comprehensible style possible.

378

379 **Results**

380 ***Transient bract formation during the floral transition is common among A. thaliana*** 381 ***accessions***

382 The typical *Brassicaceae* flower has no bract. Yet, a number of *Brassicaceae* species produce
383 some bracteate flowers (i.e. with bracts) at the base of their flowering branch (a raceme) (**Fig.**
384 **1A** and **Fig. S1 A-D**), a common botanical trait in this family (Endress 2006; German et al. 2023).
385 Hence, bract loss in *Brassicaceae* is incomplete, showing a reduction trend compared to its
386 sister clade *Cleomaceae* (**Fig. 1A**, **Fig. S1E**). Mapping the presence of bracts among the
387 *Brassicaceae* phylogeny does not reveal an evolutionary scenario (**Fig. S1A**). Some species
388 display bracts up to the first half of the raceme (e.g. **Fig. 1A**), while others only have one or two
389 bracts at the base (**Fig. S1 C, D**), e.g. in (Al-Shehbaz 2015). While the reference *Col-0* accession
390 of *A. thaliana* bears no bracts, we discovered that the natural *Tsu-0* accession displays bracts on
391 the first one to five flowers of the raceme (**Fig. 1A**). Under a stereomicroscope, a gradient of
392 bract outgrowth is visible, from fully developed bracts resembling the cauline leaves associated
393 to branches, up to filamentous, short rudimentary structures (**Fig. 1B**). Using scanning electron
394 microscopy, a mild swelling of the peduncle basis could sometimes be observed in *Tsu-0*, unlike
395 *Col-0* smooth peduncle (**Fig. 1C**). In *Tsu-0*, bract development can thus be released to varying
396 degrees in the first flowers of a branch, which we have termed “basal bracts”. Other genetically
397 diverse natural accessions produce basal bracts, albeit at a highly variable rate (**Fig. 1D**) and
398 without any clear correlation to geographic or genetic origins (**Fig. 1D**, **Fig. S1 F, G**).
399 Interestingly, *Col-0* also produces basal bracts at a low frequency, typically on lateral branches.
400 In general, we observed that basal bracts can develop at every flowering branch but with a

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401 variable frequency, even within an accession and in fixed environmental conditions. To quantify
 402 basal bracts, we

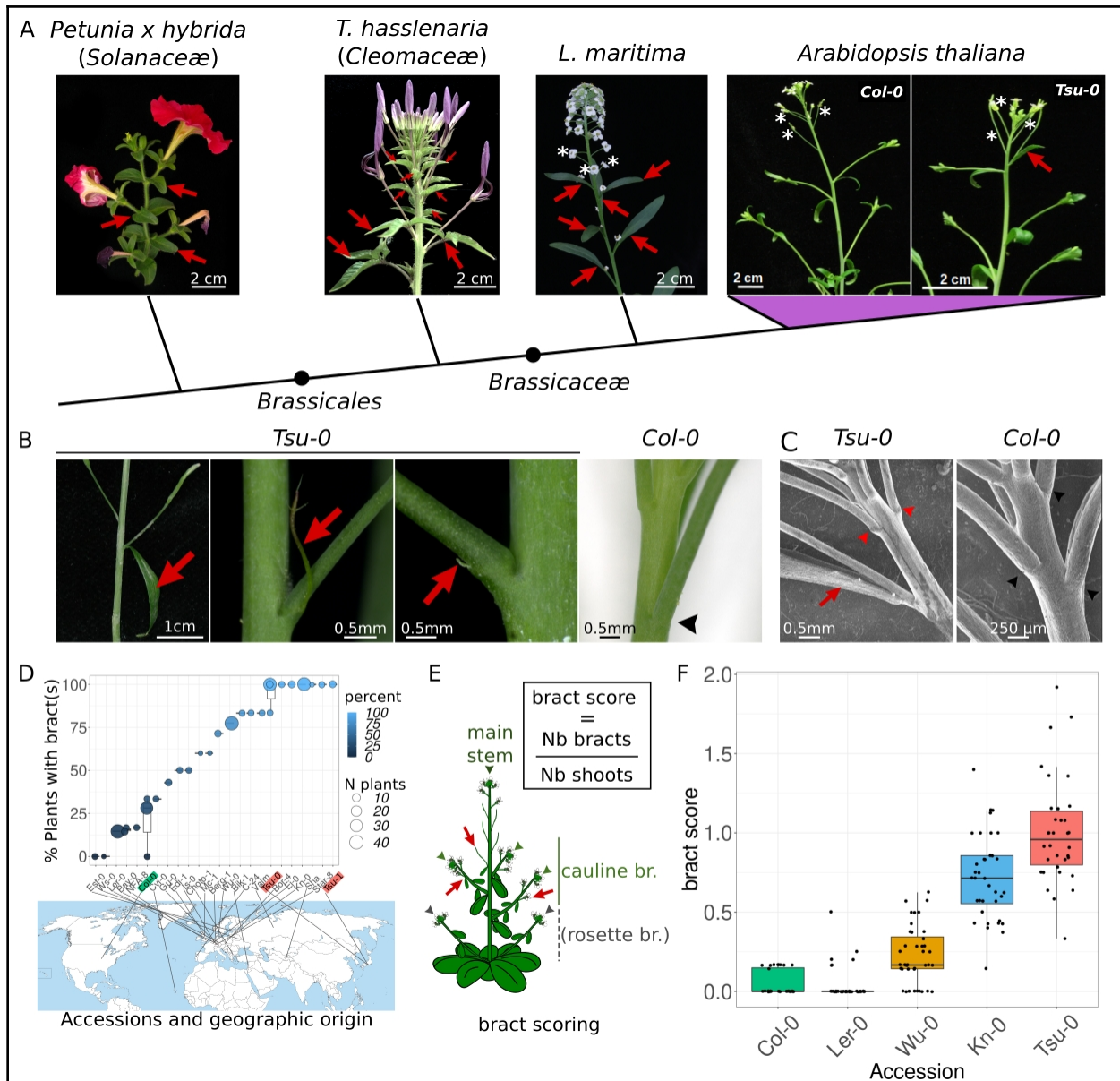


Figure 1: The presence of basal bracts is a common, natural trait in *Arabidopsis thaliana* with quantitative variations among genetic backgrounds. **A**, Examples of bracts (red arrows) in different angiosperms. *Brassicaceae* are mostly bractless but some species retain bracts at the base of inflorescence branches (e.g. in *L. maritima*). In *A. thaliana*, some natural accessions display basal bracts (e.g. *Tsu-0*) while others do not (e.g. *Col-0*). Schematic phylogenetic relationships are indicated with a cladogram below the pictures. White stars: first ebracteate flowers following previous bracteate flowers **B**, (from left to right) *A. thaliana*'s basal bracts can be true leaves or just small rudimentary filamentous structures at the base of the floral pedicel (red arrows). These structures are absent in younger flowers, as in the reference *Col-0* accession (rightmost panel, black arrowhead). **C**, Details of basal

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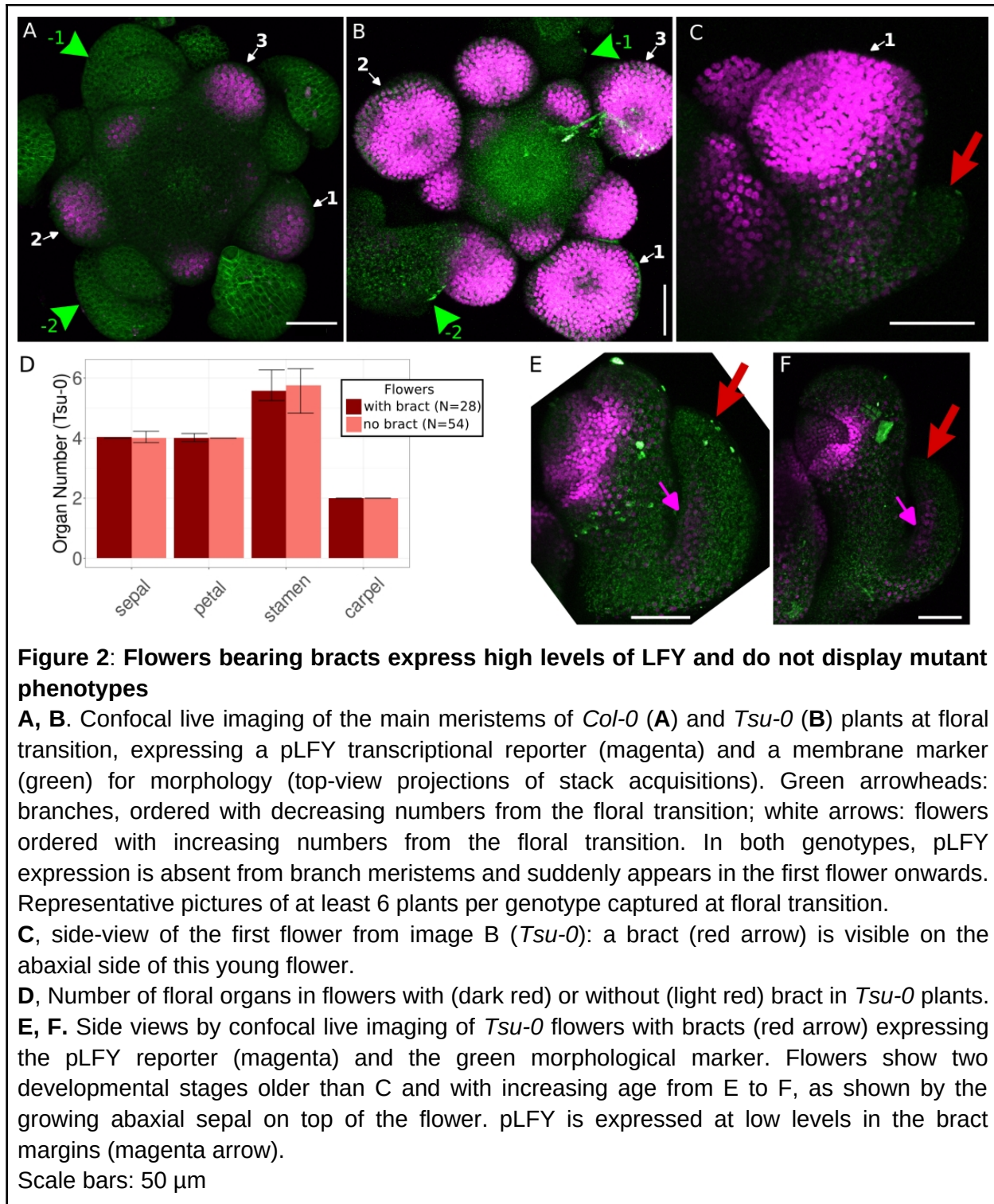
bracts by scanning electron microscopy in a *Tsu-0* inflorescence tip (left panel), showing a bract on the first flower (red arrow) and swollen base of pedicels on the two following flowers (red arrowhead). Right panel: pedicels of *Col-0* plants do not show such structures (black arrowhead). **D**, Occurrence of basal bracts in different accessions, assessed by the percentage of plants with at least one basal bract in the inflorescence. Each dot is the average value of several plants (number indicated by dot size) of a scoring assay. A box plot indicates several assays per line and thicker horizontal black lines are the median value of all scoring assays (range 1-3) in the accession. *Col-0* and *Tsu* are highlighted in green and red, respectively. The geographical origin of each strain is located in the world map below. **E**, Definition of a quantitative bract score for a single plant (see main text for detail). **G**, Quantification of basal bracts in different accessions using the bract score.

403 defined a bract score (**Fig. 1E**) which captures both intra- and inter-genotype variations (**Fig.**
404 **1F**). *Tsu-0* and *Col-0* ranked respectively as high and low bract producers and were kept for
405 further investigation.

406 ***Basal bracts develop with wild-type flowers and differ from known mutant bracts***

407 To investigate basal bract development, we imaged the emergence of the first flower. Scanning
408 electron microscopy revealed a sharp floral transition in *Col-0*: the last lateral meristem
409 subtended by a cauline leaf is immediately followed by ebracteate (i.e. bract-less) flowers (**Fig.**
410 **S2A-B**). In *Tsu-0*, the first flowers produced after the last branch bear bracts or rudimentary
411 bracts (**Fig. S2C-D**). Their association with young flowers indicates that these bracts are not
412 secondary outgrowth from mature floral peduncles. In bract-making species like the sister clade
413 *Cleomaceae*, we observed that bract emerges before the flower (**Fig. S2E**). In *A. thaliana*, the
414 abaxial position of bracts and their precocious development suggest that they are true de-
415 repressed bracts. Given LFY's central role in suppressing bract development in *A. thaliana*, we
416 question whether bracts form due to LFY perturbation at floral transition. Using a pLFY reporter
417 line, we observed in both genotypes the same sharp activation of LFY transcription from the
418 first flower onwards (**Fig. 2A-B**), regardless of bract presence in *Tsu-0* (**Fig. 2B-C**). Accordingly,
419 *Tsu-0* bracteate flowers were wild-type, with a normal number of floral organs (**Fig. 2D**). This
420 contrasts with reported bract mutants which presented severe phenotypes, such as loss of
421 floral organs or lack of determinacy with branched flowers (**Fig. S3**). Interestingly, pLFY was also
422 activated in the bract margins, albeit at lower

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423 levels than in the floral meristems (Fig. 2E-F), contrary to the proposed role of LFY as a bract
424 inhibitor. To better understand LFY contribution to basal bracts, we then re-examined bracts in
425 mutants with lowered LFY expression: *lfy*, *ufo* (Hepworth et al. 2006) and *puchi x bop1 x bop2*

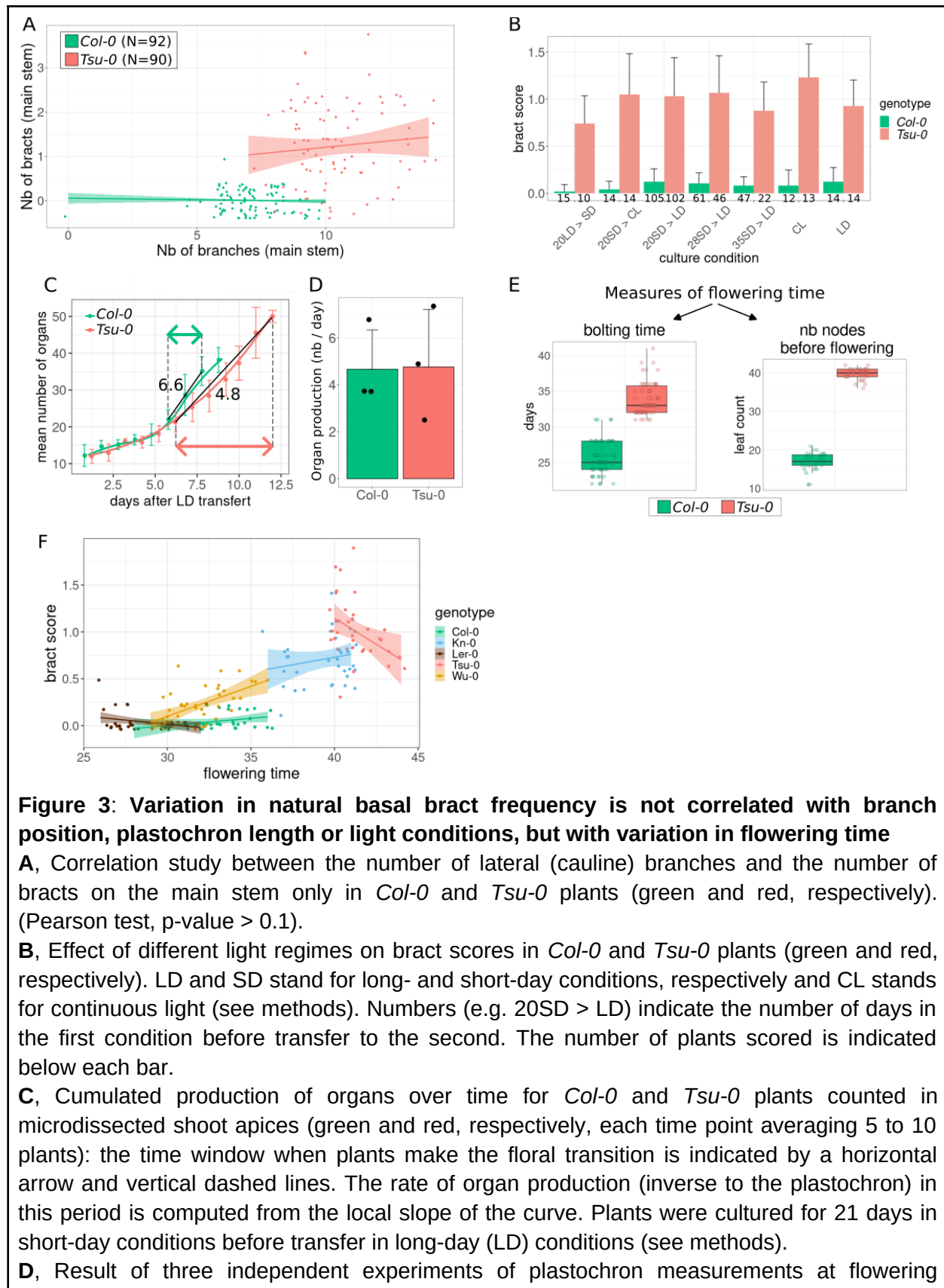
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426 (Karim et al. 2009). As previously reported (Chahtane et al. 2018), bracts in these mutants were
427 at the tip of old branches and more frequently on secondary than on main shoots (**Fig. S4 A-G**).
428 In *Tsu-0*, however, basal bracts were limited to the first flowers at floral transition (**Fig. S4H**),
429 with the same frequency on each shoot (**Fig. S4I**). In addition, at floral transition, these mutants
430 produced branches typically lacking cauline leaves, called I* branches in the literature (Ratcliffe
431 et al. 1998), which have been interpreted as flower-to-shoot transformations. The first
432 determinate flowers coming after I* branches were also ebracteate (**Fig. S4 B, C**). Hence, at
433 floral transition, loss of FMI gene function is sufficient to lose floral fate but not to restore leaf
434 development, unlike at branch tips, suggesting *LFY* effect on bract formation is context-
435 dependent. We further tested the effect of intermediate *LFY* expression levels by scoring basal
436 bracts in *LFY//lfy-null* heterozygous plants (**Fig. S4J**). The meristem was sensitive to halving the
437 genetic dose of *LFY*, as shown by the slight increase in the number of branches. However, all
438 basal flowers were wild type and ebracteate, confirming that this range of variation in *LFY* levels
439 does not affect basal bract formation. Other bract mutants rarely produce bracts at the base of
440 inflorescence but either on each node (e.g. in *JAGGED* gain-of-function mutant) or at the tip of
441 branches (**Fig. S5**). Although *tfl1* mutants produce frequent basal bracts, these are likely cauline
442 leaves that initially subtended branches, which were later transformed into flowers, as
443 suggested by the presence of a few lateral branches (**Fig. S5**). These phenotypic differences
444 suggest the genetics of bract development in mutants differ from those in *Tsu-0*, which may be
445 influenced by the particular context of floral transition. This first report of bracts associated
446 with wild-type flowers in *A. thaliana* illustrates that flower and bract formation are not
447 necessarily incompatible in this species, questioning the underlying developmental
448 mechanisms.

449 ***Basal bract frequency is unaffected by variation in photo-induction or plastochron***
450 ***length, but shows complex correlations with flowering time***

451 To characterise factors governing basal bract formation, we evaluated the effects of photo-
452 inductions as reported by (Hempel and Feldman 1995; Hempel et al. 1998). In these seminal
453 experiments, basal bracts were induced together with graded phenotypes of chimeric

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transition (see also fig. S5).

E, Differences in flowering time between *Col-0* and *Tsu-0* accession measured as bolting time in days (left) or as the number of vegetative nodes (rosette leaves and cauline leaves/branches) before the first flower on the main stem. Plants grown in LD conditions, N = 58 plants per genotype in two independent replicates.

F, Correlation study between the bract score and the flowering time (assayed when the first flower opens) in different accessions, labelled with different colours. Each dot is a plant and a linear regression standard deviation is computed for each accession.

454 shoot-flowers in natural accessions. These results lead to the “conversion” model, where a
455 strong photo-induction converts a young branch meristem (already subtended by a leaf) into a
456 bracteate flower. However, in *Tsu-0*, we never observed such shoot-flowers chimaeras (**Fig. 1, 2**
457 **and S2**) nor a negative correlation between the numbers of bracts and cauline branches (**Fig.**
458 **3A**), supporting that *Tsu-0* bracts are not linked to shoot-to-flower conversions. While Hempel
459 and Feldman observed flower formation in hours after their strong photo-induction, our floral
460 transition occurred at least one week after transfer to long days (**Fig. 3C**), suggesting milder
461 photo-inductive signals in our growth conditions. However, further varying photo-inductive
462 conditions did not affect the bract score in *Tsu-0* nor in *Col-0* (**Fig. 3B**). In the conversion
463 hypothesis, bract formation is promoted by shorter plastochrones, the time between two
464 lateral meristem initiations. Indeed, young meristems can be converted into flowers during a
465 short time window: with short plastochrones, young branch meristems sensitive to floral
466 conversion are frequently produced. In addition, the *plastochron1/2/3* mutants in rice have a
467 shorter plastochron (Miyoshi et al. 2004; Kawakatsu et al. 2006; Kawakatsu et al. 2009) and
468 make bracts. We evaluated precisely the plastochrones of *Col-0* and *Tsu-0* plants during the
469 floral transition by micro-dissecting shoot apices in time series. Despite the variability, we found
470 no consistent shorter plastochron in *Tsu-0* compared to *Col-0* (**Fig. 3C, D; Fig. S6A**). A clear
471 difference between *Tsu-0* and *Col-0* is their flowering time, which happens in older *Tsu-0* plants
472 in absolute time or in developmental time, and consistently across conditions (**Fig. 3E and Fig.**
473 **S6B**). A panel of five accessions showed a global positive correlation between flowering time
474 and bract score when comparing genotypes (**Fig. 3F**). However, within an accession, the
475 correlation can be negative (e.g. in *Ler* and *Tsu-0*), suggesting more complex relationships with

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476 flowering time. Altogether, our results indicate that basal bract formation in *Tsu-0* is not linked
477 to variation in light or productivity (plastochrone) but may be influenced by flowering time.

478 ***Basal bract development is controlled by multiple QTLs suggesting new genetic***
479 ***mechanisms***

480 To identify the genetic determinism of basal bracts, we conducted quantitative genetics using
481 crosses between *Tsu-0* and *Col-0*. F1 progeny showed intermediate bract scores while F2 bract
482 scores spread within the parental range, with a distribution that does not indicate a simple
483 monogenic trait (**Fig. 4A and Fig. S7A**). Bulk Segregant Analysis (BSA) with mapping-by-
484 sequencing identified four quantitative trait loci (QTL): two on chromosome 1 and two on
485 chromosome 5 (**Fig. S7B, C**). The large spread of F1 phenotypic values constrained F2 plant
486 selection with few plants in the high-score bulk and possibly spurious homozygous in the low-
487 score bulk. These two limits may explain the large intervals obtained. To overcome the
488 uncertainty on the phenotypic value attributed to an F2 genotype, we then used Recombinant
489 Inbred Lines (RIL), which allowed averaging of bract scores across isogenic plants. Using an
490 existing RIL set (Simon et al. 2008), we measured the average bract scores of several lines (**Fig.**
491 **S8A**) for which we checked the absence of recombination bias (**Fig. S8B**). The heritability of
492 bract score was high among RIL (65.45% +/- 1.7) and single QTL scanning mapped four peaks:
493 two most significant on chromosome 1 (named 1a and 1b) and two less significant on
494 chromosome 5 (named 5a and 5b; **Fig. 4B**). Consistent with the BSA result, this analysis
495 provided slightly different QTL positions and shorter intervals. A two-QTL scan suggested
496 additive effects between QTLs 1a and 1b (**Fig. S8C**). To confirm and reduce the intervals of these
497 two major QTLs, we used two RILs identified as heterozygous in a region overlapping with these
498 QTLs at F6 generation (**Fig. 4C-H**). F8 plants from these RIL were re-genotyped at a higher
499 density with new genetic markers (**Fig. S9A, B**) to select recombining intervals homozygous for
500 either allele of the two parents (**Fig. 4D, G**). Selected plants founded new lines forming a
501 heterologous inbred family (HIF) with identical genotypes except in a small interval, which
502 allows testing the effect of alleles in that region only. Scoring bract scores among HIF confirmed

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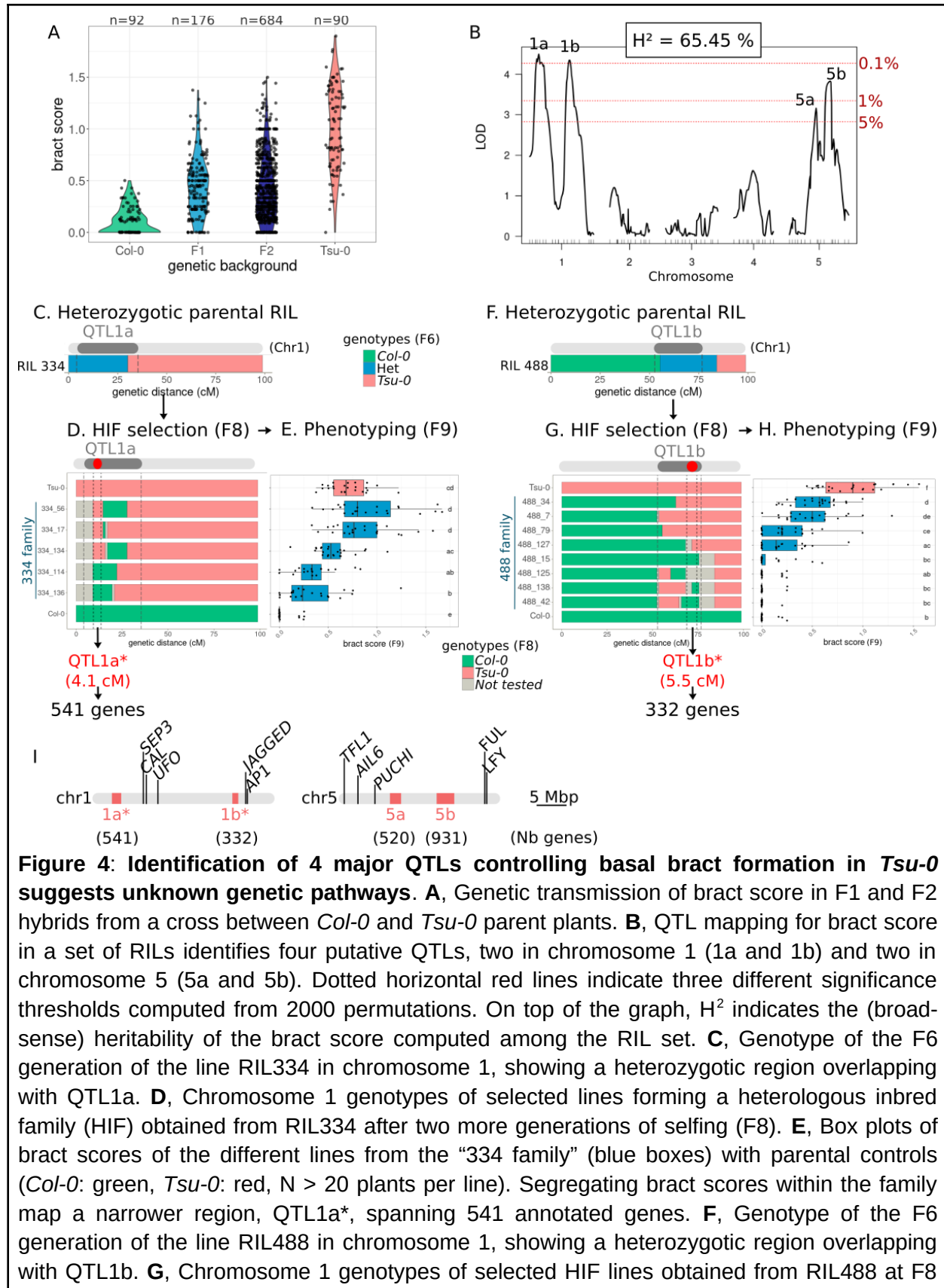


Figure 4: Identification of 4 major QTLs controlling basal bract formation in *Tsu-0* suggests unknown genetic pathways. **A**, Genetic transmission of bract score in F1 and F2 hybrids from a cross between *Col-0* and *Tsu-0* parent plants. **B**, QTL mapping for bract score in a set of RILs identifies four putative QTLs, two in chromosome 1 (1a and 1b) and two in chromosome 5 (5a and 5b). Dotted horizontal red lines indicate three different significance thresholds computed from 2000 permutations. On top of the graph, H^2 indicates the (broad-sense) heritability of the bract score computed among the RIL set. **C**, Genotype of the F6 generation of the line RIL334 in chromosome 1, showing a heterozygotic region overlapping with QTL1a. **D**, Chromosome 1 genotypes of selected lines forming a heterologous inbred family (HIF) obtained from RIL334 after two more generations of selfing (F8). **E**, Box plots of bract scores of the different lines from the “334 family” (blue boxes) with parental controls (*Col-0*: green, *Tsu-0*: red, $N > 20$ plants per line). Segregating bract scores within the family map a narrower region, QTL1a*, spanning 541 annotated genes. **F**, Genotype of the F6 generation of the line RIL488 in chromosome 1, showing a heterozygotic region overlapping with QTL1b. **G**, Chromosome 1 genotypes of selected HIF lines obtained from RIL488 at F8

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and (H) a box plot of their bract scores (blue boxes), with parental controls (*Col-0*: green, *Tsu-0*: red, N > 21 plants per line). This maps a narrower region, QTL1b*, spanning 332 annotated genes. In E and H, lines not sharing the same letter(s) are statistically different (posthoc Tukey analysis with 0.05 sign. level from a glm of bract scores fitted with a quasi-poisson distribution). I, Final genetic mapping of the 'basal bract' trait in *Tsu-0* and the location of genes reported to impact bract development in previous mutant studies.

503 both QTLs and mapped them to smaller intervals named 1a* and 1b*. Additive effects of QTL 1a
504 and 1b appeared when *Tsu-0* allele in 1b* was combined with *Col-0* allele in 1a*, the bract
505 score reaching about half of the *Tsu-0* parent bract score (see in 488 families, **Fig. 4G, H**).
506 However, *Tsu-0* parent score is tied only when both QTLs bear *Tsu-0* alleles (see in 334 families,
507 **Fig. 4D, E**). As a control, we generated a HIF to test the region between 1a and 1b and
508 demonstrated that it did not influence the bract score (**Fig. S9C-E**). However, mapped intervals
509 remained large (4.1 to 11.8 cM), containing many genes (332 to 931, 2324 annotated genes for
510 the four QTL, **Fig. 3I** and **Table S1**). The parental accessions that we re-sequenced differ by over
511 770,000 small genomic variations (SNPs and indels), with more than one variation every 175 bp
512 on average, leaving few invariant genes to exclude (**Table S1**). Interestingly, known bract-
513 related genes were absent from the QTLs (**Fig. 4I**). However, a careful RIL phenotype inspection
514 provided additional hints into the developmental pathways possibly involved in *Tsu-0* basal
515 bract formation. First, we observed that the cauline branch number positively correlates with
516 the bract score and QTL1b overlaps with a QTL controlling cauline branch number (**Fig. S10**).
517 This suggests that the gene controlling basal bracts in QTL 1b* might also promote more cauline
518 branches. Second, two transgressive, partially penetrant phenotypes appeared in the RILs: the
519 bract position often shifted to a mid-peduncle position (**Fig. S11, A-F**) and signs of incomplete
520 floral determinism (mostly branched flowers) were observed (**Fig. S11, N, O**). The shifted bract
521 position resembles flower-preceding prophylls in some natural species (Endress 2006; Prenner
522 et al. 2009). Interestingly, this phenotype is frequent in certain bract mutants like *soc1*, *tfl1* and
523 even systematic in *bop1 x bop2* (**Fig. S11 H-M**). However, this trait did not correlate with any
524 specific allelic combinations among the HIF families (**Fig. S11G**). Indeterminism in HIF 488 was
525 associated with *Tsu-0* QTL 1b* and *Col-0* haplotypes in other mapped QTLs (**Fig. S11P**). Other
526 indeterminism cases occurred in HIF 334, but without consistent genetic combinations of

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527 mapped QTLs. Such transgressions suggest that complex genetic interactions may be required
528 in *Tsu-0* to ensure proper bract development and positioning with a wild-type flower. To
529 conclude, our data demonstrate that the genetic control of basal bracts in *Tsu-0* is complex and
530 relies on several genes having additive effects on bract development and epistasis on other
531 traits.

532 ***Transcriptomics of the floral transition suggests new pathways associated with bract***
533 ***development***

534 To identify genes whose expression changes with bract development, we profiled meristem
535 transcriptomes over the floral transition in both accessions. Microdissected meristems were
536 matched to the same four developmental stages: vegetative, late vegetative, transition and
537 floral (V, L, T and F, respectively, **Fig. 5A**). This ensured that, despite different absolute
538 flowering times (**Fig. S12A**), the transcriptomes of the two accessions were realigned using the
539 floral transition as a common developmental clock. After RNAseq, principal component analysis
540 (PCA) validated replicate consistency (**Fig. S12B**) and the precision of the staging was confirmed
541 by the synchronised expression between accessions of genes such as *FT*, *LFY* or *AP1*, key
542 regulators of floral transition and flower identity (**Fig. 5B**, see also **Fig. S12C** for other reference
543 genes). For *LFY*, this RNAseq data supported our results from the p*LFY* transcriptional reporter
544 lines (**Fig. 2**) and genetic studies (**Fig. S4**), indicating that *LFY* is not involved in *Tsu-0* basal bract
545 development. The stage T stood out as a critical transition, with the highest number of gene
546 expression changes in both accessions (**Fig. 5C**). At stage T, *Col-0* and *Tsu-0* meristem
547 transcriptomes also diverged the most, with up to 4,759 genes differentially expressed (DE)
548 (**Fig. 5D**). Bract and flower initiations start before any morphological event (Heisler et al. 2005).
549 Given the delay between stages T and F (a median of 1 to 2 days, **Fig. S12A**) and the
550 plastochron (**Fig. 3B, C**), the first flowers and their bracts must be initiated at stage T. Thus,
551 transcriptional changes associated with bract formation in *Tsu-0* should transiently appear at
552 stage T and progressively fade by stage F since only the 1 to 5 first flowers present a bract (**Fig.**
553 **1C, Fig. S4H**).

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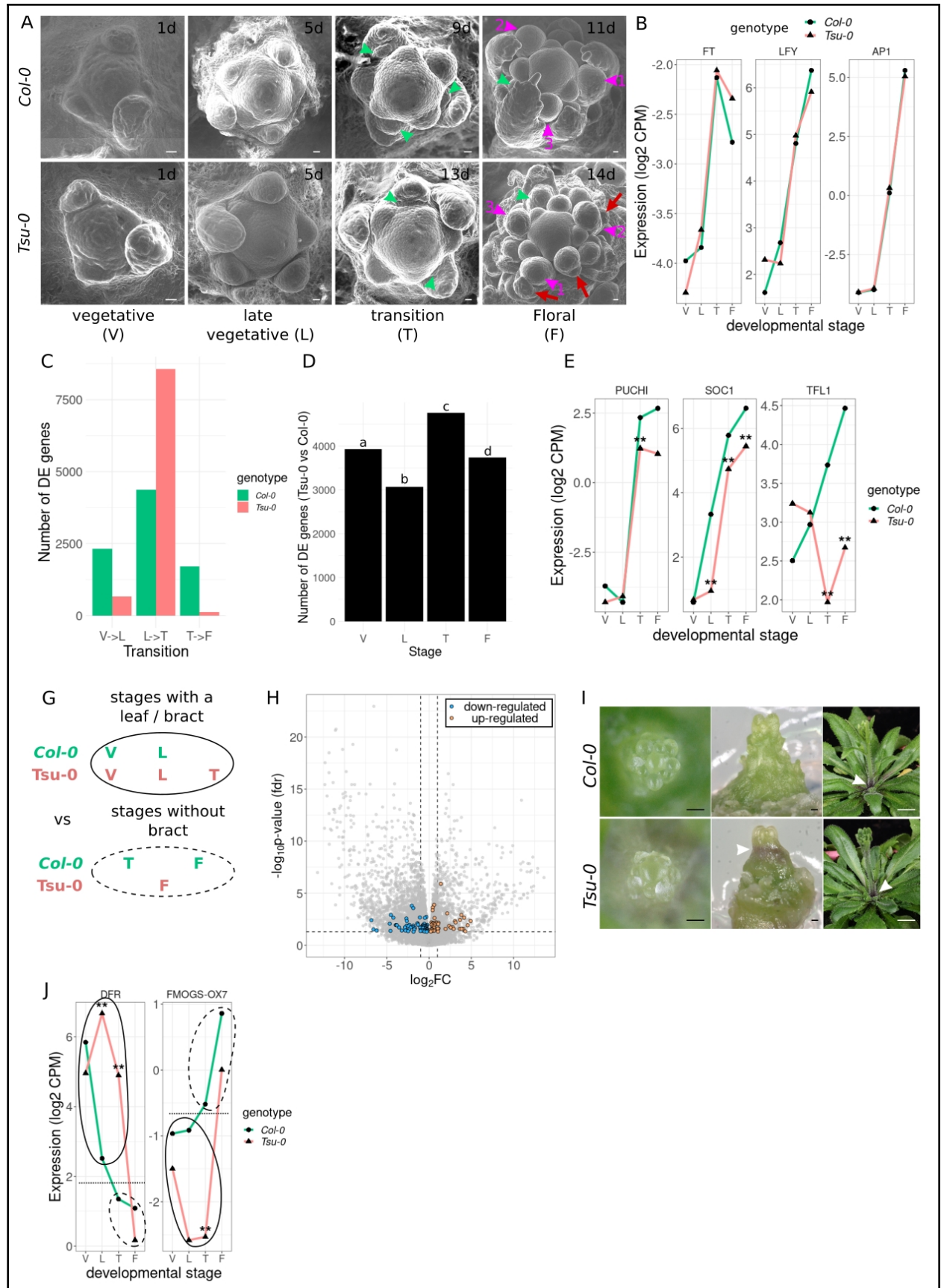


Figure 5: Transcriptomic divergence between *Tsu-0* and *Col-0* meristems peaks at the
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554 Since GO terms yielded no informative clues from the high number of DE genes at stage T (**Fig.**
555 **S14A**), we first examined whether known bract-related genes could be affected, although we
556 knew from our mapping that they cannot be at the origin of basal bracts in *Tsu-0* (**Fig. 4I**). Only
557 *PUCHI*, *SOC1* and *TFL1* significantly differed at stage T between *Tsu-0* and *Col-0* (**Fig. 5E** and **Fig.**
558 **S12D**) but these differences persisted until stage F even though bracts disappeared with older
559 flowers, suggesting that the variation of these gene expressions is not essential in bract
560 formation.

561 To discover genes without *a priori*, we then performed a comparative transcriptomic approach
562 with additional mutant data. Since *lfy* and *puchi x bop1 x bop2* mutants stop making leaves at
563 floral transitions (I* branches, **Fig. S4B, D**) while *jagged-5d* plants always produce a bract,
564 transcriptomic cues for bract development may only be present in *jagged-5d* meristems and
565 not in the other two backgrounds. As expected from their genetic background and
566 developmental stage, these mutants clustered with *Col-0* stage F in a PCA (**Fig. S12B**). By
567 comparing DE genes of each mutant with *Col-0*, DE genes specific to *jagged-5d* were isolated
568 (**Fig. S13A**). This set revealed an enrichment for biological processes related to shoot, phyllome
569 development and photosynthesis, possibly linked to persistent bract formation (**Fig. S13B**). Only
570 413 of these genes were shared with DE genes of *Tsu-0* at stage T, with Go terms pointing again
571 to chloroplast functions and less expectedly, to metal ion transport and homeostasis (**Fig. S13C,**
572 **D**). If one of the causative genes mapped in *Tsu-0* (**Fig. 4, Table S1**) was differentially expressed
573 (so not an allele acting post-transcriptionally) and shared with *jagged-5d*, it should be contained
574 in this short list. Cross referencing transcriptomic and QTL-mapping datasets yielded 33
575 candidate genes (**Fig. S13E, Table S2**). None of them is known to be linked with bract
576 development or with flowering in general. Although these particular genes should be validated,
577 this mutant comparison suggests that new uncharacterized genetic pathways may be involved
578 in the development of bracts.

579 In a third approach to capture genes involved in bract formation, we specifically took advantage
580 of the time-series information. Briefly, expression data were gathered in two groups
581 corresponding to either bract-less (*Col-0* T, F, and *Tsu-0* F) or leaf/bract-producing meristems
582 (the other samples, including *Tsu-0* T) and we selected genes whose expression profile clusters

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583 these two groups apart (**Fig. 5G**). Only 124 genes met the criteria of such “putative bract
584 regulators” (**Fig. 5H, Table S3**), including *SOC1*, as a putative negative regulator. Just 19 genes
585 overlapped with the genes also DE in *jagged-5d* (**Fig. S13C, Table S3**), without pointing to
586 particular biological functions. Instead, two enriched GO-terms emerged from these putative
587 bract regulators: anthocyanin biosynthesis and salicylic acid (SA), associated with up- and
588 down-regulated genes, respectively (**Fig. S14D-F**). Backtracking these ontology terms for all DE
589 genes at stage T (**Table S4 and S5**) retrieved more genes associated with these pathways,
590 supporting that their activity levels differ between *Tsu-0* and *Col-0* at the critical stage T (**Fig.**
591 **S14I**). High anthocyanin production in *Tsu-0* at stages L and T was evident from the frequent
592 purple coloration just below the meristems, contrasting with the green tissues in *Col-0* (**Fig. 5I**).
593 Sometimes, this purple extended to young organs where bracts are initiated (**Fig. 14J**). As the
594 stem grew, the pigment receded to the rosette junction in both accessions (**Fig. 5I**). This
595 transient anthocyanin presence specific to *Tsu-0* supported our clustering strategy for capturing
596 genes involved in transient bract development during flowering transition. Interestingly, among
597 the 124 expression-based putative bract regulators (**Fig. 5H**), 12 also lied in the mapped QTLs
598 (**Fig. 4I**). They all differed from the 33 genes selected before via specific overlap with *jagged-5d*
599 (**Table S2**). Interestingly, we found one anthocyanin biosynthetic enzyme (the dihydroflavonol
600 reductase, DFR) and five SA-responding genes (see **Table S3**). For example, **Figure 5J** shows the
601 expression profiles of two such genes. Further work is required to test whether these
602 candidates contribute to basal bract formation in *Tsu-0* and if the anthocyanin and/or SA
603 pathways are involved in this natural variation. However, our transcriptomics exclude most of
604 the genes previously associated with bract development in mutants, and suggest instead new
605 candidate pathways promoting bract outgrowth with a wild-type flower during floral transition.

Bract development occurs in a time window when many genes are desynchronized

607 Our clustering approach (**Fig. 5G**) tended to select genes whose transcriptional dynamics were
608 delayed in *Tsu-0* compared to *Col-0* (**Figure 5J**), with changes of RNA levels occurring later in
609 *Tsu-0*. This defines a transcriptional heterochrony. Since bracts have been sometimes
610 considered as a heterochrony because the juvenile leaf trait is maintained at older stages, we
611 decided to characterise the extent of changes in the timing of gene expression between the two

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612 accessions. We first plotted the two main axes of variance from a PCA after grouping samples
613 by genotype and time points (**Fig. 6A**). While the second axis of variance (~24.4%) differentiates
614 genotypes, the main axis (47.9% of variance) orders the sampling time points chronologically in
615 both genotypes, representing a transcriptomic age. Surprisingly, *Tsu-0* stage T does not align
616 with *Col-0* stage T on this axis, clustering instead with the stages F: this transcriptome is closer
617 to a stage F, explaining the limited expression changes when progressing to the next stage F
618 (**Fig. 5C**).

619 As suggested by (Calderwood et al. 2021), the transcriptomes of two genotypes during
620 flowering cannot be aligned to a single developmental time; each gene may desynchronize
621 differently, sometimes in opposite directions. To quantify gene desynchronization between *Col-*
622 *0* and *Tsu-0*, we leveraged our previous approach of curve registration (Calderwood et al. 2021)
623 to predict subtle temporal shifts of gene expression in our dataset (Kristianingsih 2024). This
624 shift is relative to the floral transition, used as the common reference clock between the two
625 accessions. Thus, genes with no shift, like *AP1* (**Fig. 5B and Fig. 6B**), may still be shifted in
626 absolute time, because *Tsu-0* plants flower later with an older absolute age. Positive and
627 negative shifts reflect desynchronization relative to the event of floral transition: gene
628 expression dynamics may shift earlier or later. Unlike our previous clustering approach, this
629 registration method uses a common scaled expression level and computes only temporal shifts,
630 regardless of absolute expression levels (**Fig. 6B and Fig. S15A**). Few genes could not be
631 registered (**Fig. 6B**, $N = 43$), suggesting that most genes follow similar temporal dynamics in
632 both accessions (see examples **Fig. S12C, D or Fig. 5B, E**). Most genes were classified into three
633 categories: null, negative and positive shifts (**Fig. 6B**). A null shift indicates that the gene
634 expression in *Tsu-0* stays “in phase” with flowering, like *AP1* (compared with **Fig. 5B**). A positive

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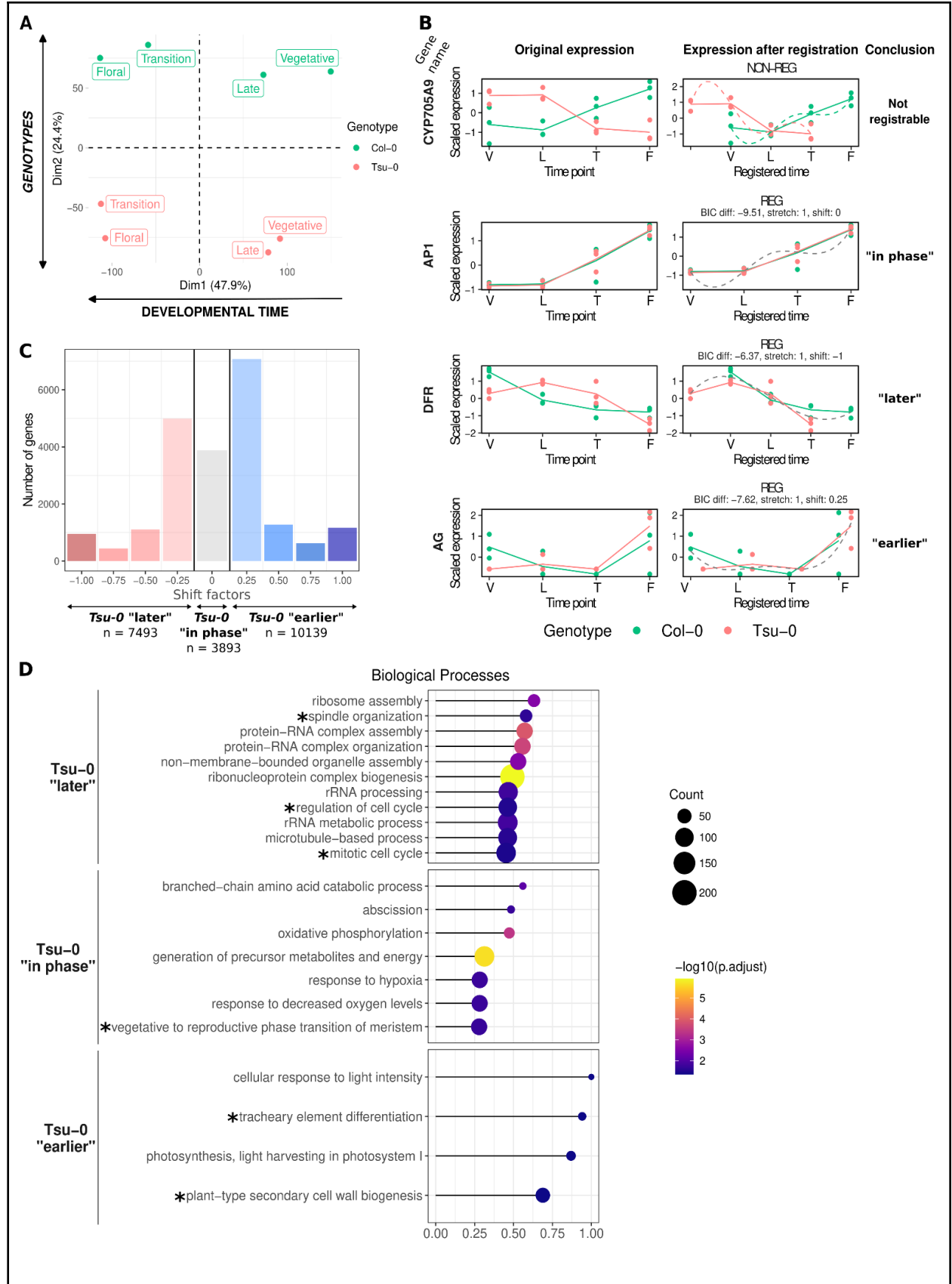


Figure 6: Massive and complex transcriptional desynchronisation coexist in *Tsu-0* across the floral transition.

A, PCA of RNAseq data from microdissected meristems of *Col-0* and *Tsu-0* over the four sampled stages (biological replicates averaged per time points). The two main axes of the PCA can be interpreted as developmental time and genotype, respectively.

B, Examples of temporal registration of gene dynamics (right panels) between *Col-0* (green) and *Tsu-0* (red) from scaled expression levels (left panel). Dots represent the expression levels of independent biological replicates, while lines indicate the mean expression level at each time point. In the right panel, the green (or red) dotted curves represent the fitted models for *Col-0* (or *Tsu-0*) independently, while the grey dotted curve represents the joint model for both *Col-0* and *Tsu-0*. If the green and red dotted curves are used, it means the two time series are best explained by two independent models, indicating they are not similar. Conversely, if the grey dotted curve is used, it suggests that a single model best explains both time series, indicating they are similar. The name of the gene plotted is indicated on the left of each row. These four genes exemplify the four possible categories (from top to bottom, respectively): genes that cannot be registered (N = 43, e.g. *CYP705A9*), genes with identical temporal dynamics (shift = 0, e.g. *AP1*, see also Fig. 5B) and genes whose expression dynamics in *Tsu-0* must be shifted negatively (e.g. *DFR*, see also Fig. 5J) or positively (e.g. *AG*) to align with *Col-0*. The last column provides a biological interpretation of the computed shift (see main text): a null shift indicates that the expression dynamics in *Tsu-0* stay “in phase” with floral transition while negative or positive shifts indicate that the expression dynamics in *Tsu-0* are desynchronized later or earlier, respectively, than the phenotypic progression of floral transition.

C, Distribution of heterochronic shifts between *Tsu-0* and *Col-0* on the entire meristematic transcriptome, computed by the registration method from B. The shift value is colour-coded in a red-to-blue gradient from -1 to 1.

D, GO term enrichment analysis associated with the three categories of heterochronic shifts. The list of all significant ‘biological process’ GO terms (BH-adjusted p.value < 0.05) was simplified using semantic similarity (cutoff = 0.7) and the Rich Factor was computed for the remaining terms, revealing the proportion of genes involved among all the genes associated with this GO term. Dot size indicates the count of genes and the color scale is the statistical significance (BH-adjusted p.value) of the enrichment in the shift category. Stars indicate GO terms referring to developmental processes.

635 or negative shift indicates that gene dynamics in *Tsu-0* occur earlier (e.g. *AG*) or later (e.g., *DFR*,
636 see also **Fig. 5J**), relative to the floral transition, revealing heterochronies between
637 transcriptomic and phenotypic processes. Transcriptome-wide, the shifts were broadly
638 distributed, showing massive and complex desynchronisation of gene dynamics (**Fig. 6C**). The
639 floral transition in *Tsu-0* did not impose its clock to the entire meristematic transcriptome: only
640 ~18% of genes (n=3879) stayed in phase with this process while the majority showed delayed or
641 advanced expression dynamics. Even more genes shifted earlier relative to the floral transition,

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642 supporting the PCA interpretation (**Fig. 6A**) and confirming that the bract, if considered as
643 “juvenile”, did not represent the majority of heterochronies at the transcriptomic level.
644 As expected, genes in phase were primarily linked to flowering and developmental phase
645 change as shown by key regulators of these pathways (**Fig. S15B, C**), which also emerges from
646 GO term enrichment analysis (**Fig. 6D**). In contrast, known bract genes exhibit a wide range of
647 shifts, from very early to very late, suggesting that they are not collectively involved in *Tsu-0*
648 bract development (**Fig. S15A, C**). GO term analysis for each shift category highlighted the
649 particular processes desynchronized from flowering in *Tsu-0* compared to *Col-0* (**Fig. 6D**). For
650 instance, vascular differentiation (two GO terms mentioning tracheary element and secondary
651 cell wall) emerges as a process shifted earlier in *Tsu-0* relative to the floral transition (**Fig. 6D**).
652 Its dynamics may be driven by other progression factors like absolute age, suggesting its loose
653 connection with the flowering pathway. Conversely, processes shifted later in *Tsu-0* revealed
654 terms related to cell division (spindle, cell cycle, mitosis) and ribosomal biogenesis (six GO
655 terms mentioning ribosome, rRNA, ribonucleoprotein and protein-RNA complexes; **Fig. 6D**),
656 which suggests that core meristematic functions are prolonged in *Tsu-0*. Further work is yet
657 needed to explore whether the desynchronization of these processes relative to floral transition
658 impacts bract development.
659 Finally, this focus on gene desynchronisation provided an explanation to the fact that gene
660 expression diverged most at the stage T (**Fig. 5D**). The genes controlling floral transition change
661 swiftly their expression levels upon stage T when many other genes have different levels
662 between both accessions, because they are no longer synchronised with flowering in *Tsu-0* (**Fig.**
663 **S16**). A transient process such as flowering is likely to occur in varying gene expression states,
664 especially if it is fast and depends on a small number of genes. Massive desynchronization of
665 gene dynamics creates transcriptional variation, potentially leading to developmental variation
666 at the time of flowering, such as bract development. In conclusion, our work provides the first
667 analysis of transcriptome-wide meristematic heterochronies between two *A. thaliana*
668 accessions, revealing that natural bract development during floral transition is more complex
669 than a prolonged vegetative phase.
670

671 **Discussion**

672 This study documents a natural variation for the presence of bracts at the base of
673 flowering branches in *A. thaliana*, which was used to investigate their genetic and
674 developmental basis. Comprehensive phenotypic characterizations indicate strong differences
675 with known mutant bracts and similarities with bracts of bracteate species. Combining
676 quantitative genetics, genomics and meristem transcriptomics on the accession *Tsu-0*, our
677 results suggest new mechanisms controlling bract outgrowth and highlight the phenomenon of
678 massive gene desynchronizations at floral transition, raising new questions about their
679 implications in development and evolution.

680 ***A new mechanism unlocking bract development without affecting floral identity***
681 ***during floral transition***

682 We used two quantitative genetics approaches (BSA and RIL) and a transcriptomic approach to
683 identify the genetic mechanisms controlling basal bract formation in *Tsu-0*. We identified at
684 least four major QTLs, the two main on chromosome 1 with additive effects (**Fig. 4** and **Fig. S7**).
685 However, the density of polymorphisms between the two accessions and the high number of
686 differentially expressed genes resulted in many potential candidates within the mapped
687 intervals. Further fine-mapping or a GWAS analysis on a larger accession panel could provide a
688 higher resolution and even reveal more causative variations.

689 Our work also proposes new pathways for bract development: genetic interactions between
690 *SOC1*, *TFL1* and *PUCHI* (**Fig. 5E**), chloroplast or metal ion transport and homeostasis (**Fig. S13D**),
691 anthocyanin biosynthesis and response to salicylic acid (**Fig. 5I, J**; **Fig. S14E-J**), ribosome
692 biogenesis (**Fig. 6D**). The low number of transcription factors (**tables S1, S2, S3**) compared to
693 enzymes and genes related to metabolic pathways (**Fig. 5 and 6**; **Fig. S13 and S14**) aligns with
694 studies reporting that genes with basic metabolic and cellular functions control specific
695 developmental processes in plants (Tsukaya et al. 2013). Likewise, the *PLA1/2/3* genes in
696 *Poaceae* are three unrelated metabolic enzymes (a cytochrome P450, MEI2-like RNA-binding
697 protein and a glutamate carboxypeptidase II, respectively) that partially suppress bract
698 outgrowth in a redundant manner through a still unknown mechanism (Kawakatsu et al. 2009;

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699 Wang et al. 2021). Our data may thus be helpful to investigate the mechanisms that control
700 bract development.
701 Despite the remaining uncertainty about the causal genes and pathways, our data show the
702 existence of a new bract developmental process different from regained bracts produced in
703 mutants. *Tsu-0* basal bracts display specific features: association with wild-type flowers (**Fig. 2**
704 and **Fig. S2, S3**); wild-type bract shape and position (**Fig. 1, Fig. S11**); presence restricted at the
705 base of the raceme (**Fig. S4, S5**); no modification of plastochron rate (**Fig. 3B, Fig. S6A**);
706 independence from light regime (**Fig. 3D**). Not only such phenotypes differ from those reported
707 in bract mutants, but none of the known “bract mutant genes” were found in QTL intervals,
708 indicating that the causal polymorphisms involve other genes and that bract development could
709 be de-repressed without affecting floral meristem identity. However, because of the
710 transgressive indeterminism observed in some RILs (**Fig. S11**), it cannot be ruled out that some
711 of the bract causal genes could also affect flower development, but genetic interactions would
712 suppress these floral phenotypes while maintaining the basal bracts.

713 ***Transcriptomic heterochronies at floral transition may challenge developmental***
714 ***canalization***

715 The transient formation of bracts at the base of each raceme raises many biological questions.
716 This trait is widespread within the *Brassicaceae* phylogeny (**Fig. 1S**). It is also known for decades
717 that several species within their natural context display developmental variations at the base of
718 the raceme, including bracts, flower-to-shoot transformations, or flower dimorphisms (Arber
719 1931a; Arber 1931b). Since these basal nodes are produced just at the floral transition, this
720 suggests that developmental canalization (ensuring an invariant phenotypic output) is less
721 effective at this stage. The variability of bract formation from branch to branch and plant to
722 plant (**Fig. 1 D-F, Fig. 4A**) also suggests limited developmental canalization. Our data show that
723 natural genetic variation is sufficient to reveal a higher frequency of phenotypic variations at
724 the base of branches. To our knowledge, no specific hypothesis has been proposed to explain
725 the lower developmental canalization at the floral transition.
726 We propose that transcriptional heterochronies may account for some of this phenomenon. In
727 bract-less species, bract development is often described as a prolonged vegetative state,

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728 characterised by the persistence of a juvenile trait (a leaf) with an adult trait (a flower) (Alberch
729 et al. 1979; Buendía-Monreal and Gillmor 2018). Using microdissected meristems at different
730 stages (**Fig. 5**), we expected to find this signature in the bract-making *Tsu-0* transcriptome.
731 Instead, we discovered that the floral transition occurs in an older transcriptome rather than a
732 younger one (**Fig. 6A, C**). In absolute time, *Tsu-0* plants flower later (**Fig. 3 E, F; Fig. S12 A**), so
733 the flowering-related genes are actually shifted later. However, a large majority of genes get
734 out of synchronisation with flowering. In absolute time, some may keep their dynamics or even
735 shift earlier, both categorised as “earlier” in our analysis using flowering as the reference, while
736 other genes can shift even later than flowering (**Fig. 6 B, C**). Confirming our previous results
737 using *Brassica rapa* cultivars and *A. thaliana* (Calderwood et al. 2021), such massive and
738 complex gene desynchronisation during floral transition seems to be a general rule at both
739 intra- and inter-species level. Flowering time is under strong selection pressure in *A. thaliana*
740 (Bloomer and Dean 2017). If selective adaptation of flowering time constantly shifts and
741 desynchronizes the flowering-related genes from numerous others, this can create new global
742 gene expression states, especially at the transition when gene expressions vary quickly (**Fig.**
743 **S16**). This could promote transient developmental variations, like basal bracts.
744 The consequence of gene desynchronization is to create a peak of transcriptome divergence
745 upon floral transition. Such a peak has been reported in *Solanaceae* where it was associated
746 with the phenotypic evolution of inflorescence complexity (Lemmon et al. 2016). Mirroring the
747 “inverse hourglass” model for animal embryogenesis, morphological variations would be
748 promoted by transcriptomic divergence during intermediate developmental steps. Hence, the
749 sensitivity of floral transition to heterochronies at the transcriptional level could have larger
750 implications for phenotypic evolution from populations to species.

751 ***Evolution of bract loss in Brassicaceae and Angiosperms and the possible role of***
752 ***heterochronies***

753 Heterochrony is often considered as a powerful mechanism for evolutionary change (Buendía-
754 Monreal and Gillmor 2018; Petrone-Mendoza et al. 2023). Here, we propose that
755 heterochronies at the floral transition may lower developmental canalization, contributing to
756 bract derepression in some accessions. Conversely, heterochronies might have been involved in

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757 bract loss in the *Brassicaceae* ancestor. Current evolutionary scenarios for bract loss are
758 deduced from mutants and do not involve heterochrony. In *A. thaliana*, flowers would have
759 evolved the ability to repress bract development (Whipple et al. 2010), while In *Poaceae*,
760 boundary regions may have acquired this function through genes like NTT (Whipple et al. 2010;
761 Xiao et al. 2022). However, direct regulation of NTT by SPL genes in maize (Xiao et al. 2022) and
762 rice (Wang et al. 2021) provide a link with a major regulator of heterochrony in plants, the
763 SPL/miR156 balance (Buendía-Monreal and Gillmor 2018). SPL genes have not been found to
764 control bract development in *A. thaliana*. Recently, *SOC1*, *FUL* and *AGL24* were shown to be
765 more effective than *LFY* or *BOP1/2* in repressing bracts (Manuela and Xu 2024 Mar 28). These
766 genes are special because they act both as FMI genes and as flowering time genes. Our data did
767 not correlate bract outgrowth with any FMI genes (**Fig. 2, Fig. 5**) while flowering time was
768 clearly impacted at the phenotypic (**Fig. 3E, F**) and transcriptional level (e.g., *FLC* in **Fig. S12C or**
769 **Fig. S15C**). Since flowering time is prone to heterochrony, this could connect bract with
770 heterochrony in *A. thaliana*. Hence, despite differences in molecular players, bract outgrowth in
771 *Brassicaceae* and *Poaceae* may share more similarities than previously described.
772 Deducing evolutionary events from mutants relies on the assumption that natural evolution
773 proceeds in the opposite direction to artificial mutants, which should be taken with caution.
774 Indeed, actual ebracteate plants have accumulated many evolutionary changes since they
775 diverged from the last bracteate ancestor: genetic interactions revealed by mutants in current
776 species may be totally irrelevant in the ancestor. It has also been proposed as the “Dollo’s law”
777 (Gould 1970) that lost traits cannot be regained and “*must be constructed afresh in some*
778 *different mode*” (Arber 1918). However, heterochronies have been proposed as a mechanism to
779 break Dollo’s law (Cronk 2009). Since ebracteate plants still produce leaves elsewhere,
780 activating or repressing this functional developmental program by heterochronies could
781 effectively coordinate bract and flower development, explaining both bract loss and their
782 “flickering presence” (Marshall et al. 1994) within and between species in the entire
783 *Brassicaceae* family. Further work, notably including different bracteate and ebracteate species,
784 will be necessary to test these hypotheses and clarify the genetic and developmental
785 mechanisms that led to bract loss in *Brassicaceae*.

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801

802 **Competing interests,**

803 The authors declare that they have no competing interests

804 **Author contributions**

805 Sana Dieudonné: Conceptualization, Investigation, Software (implementation), Formal analysis,
806 Visualization, Methodology, Writing -review and editing; Ruth Kristianingsih: Formal Analysis,
807 Methodology, Software (designing, programming, testing, implementing), Visualization, Writing
808 -review and editing; Stephanie Laine, Investigation; Béline Jesson: Formal analysis, Véronique
809 Vidal: Supervision, Rachel Wells: Supervision, Writing -review and editing; Richard Morris:
810 Supervision, Writing -review and editing; Fabrice Besnard: Conceptualization, Project
811 administration, Supervision, Investigation, Formal analysis, Methodology, Software
812 (programming, implementing), Funding acquisition, Visualization, Writing - original draft, review
813 and editing.

814 **Data availability**

815 Whole-genome DNA-seq data of the parental lines (*Col-0* and *Tsu-0*) and of the two F2 pools
816 used for bulk segregant analysis are deposited under this identifier: [doi:10.57745/Z80SIM](https://doi.org/10.57745/Z80SIM).
817 Time-course RNA-seq data of *Col-0* and *Tsu-0* micro-dissected meristems during flowering are
818 available with these doi: [doi:10.57745/DKMQ06](https://doi.org/10.57745/DKMQ06), [doi:10.57745/7JI3E7](https://doi.org/10.57745/7JI3E7), respectively. RNA-seq
819 data of micro-dissected shoot meristems of the mutant lines (*puchi-1x bop1-4 x bop2-11*, *lfy-12*
820 and *jagged-5d*) at early flowering stage are available at [doi:10.57745/HAGJJH](https://doi.org/10.57745/HAGJJH).

821

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1116 **Supporting Information**

1117 **Figures S1 to S16**

- 1118 **Figure S1:** Basal bracts are common among *Brassicaceae* tribes and their presence is not
1119 correlated to broad geographic nor genetic origin in *A. thaliana*.
- 1120 **Figure S2:** Comparing basal bract formation in *Tsu-0* with bracts forming in a bract-making sister
1121 species.
- 1122 **Figure S3:** Known bract mutants in *A. thaliana* display abnormal floral phenotypes and
1123 indeterminacy.
- 1124 **Figure S4:** Bracts of mutants impaired in floral meristem identity genes are not located at the
1125 floral transition like *Tsu-0* natural basal bracts.
- 1126 **Figure S5:** Known bract mutants in *A. thaliana* do not display bracts specifically at the floral
1127 transition.
- 1128 **Figure S6:** Plastochron variation and flowering heterochronies in *Tsu-0* versus *Col-0* accessions
- 1129 **Figure S7:** Bulk segregant analysis of basal bract formation (*Tsu-0* x *Col-0*) identifies four
1130 putative QTLs.
- 1131 **Figure S8:** Quantitative genetics of basal bracts formation in *Tsu-0* using a set of RILs with the
1132 reference accession *Col-0*.
- 1133 **Figure S9:** Finer mapping of QTLs.
- 1134 **Figure S10:** QTL1b* identified for basal bract correlates with a higher cauline branch number.

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1135 **Figure S11:** Transgressive phenotypes in RILs and their genetic determinism provide further
1136 information of the genetic pathways involved.

1137 **Figure S12:** Transcriptomic profile associated with basal bract formation

1138 **Figure S13:** Transcriptomic cross-comparisons between bract mutants and *Tsu-0* at T stage
1139 capture an enrichment for processes related to photosynthesis and provide a short list of 33
1140 causal candidate genes.

1141 **Figure S14:** GO term analysis at T stage and the identification of anthocyanin biosynthesis and
1142 SA-responding pathway enrichment among putative bract regulators.

1143 **Figure S15:** Temporal Registration of expression dynamics in *Tsu-0* over the floral transition for
1144 genes related to bract development, floral identity and floral transition.

1145 **Figure S16:** A working model for natural basal bract formation in *Arabidopsis thaliana*.

1146

1147 **Tables S1 to S8** are large spreadsheets available for download:

1148 **Table S1:** List of annotated genes lying in mapped QTLs controlling bracts in *Tsu-0*, with
1149 additional information from RNAseq and genomic variant analysis.

1150 **Table S2:** Intersection of genes differentially expressed in *Tsu-0* at stage T and specifically in the
1151 *jagged-5D* mutant meristem and mapped within *Tsu-0* bract QTLs.

1152 **Table S3:** Putative bract regulators in *Tsu-0* identified by clustering apart expression from bract
1153 and non-bract making stages.

1154 **Table S4:** Details for all genes associated with “response to salicylic acid” about their expression
1155 at stage in *Tsu-0* and their identification as putative bract regulators.

1156 **Table S5:** Details for all genes associated with anthocyanin metabolism about their expression
1157 at stage in *Tsu-0* and their identification as putative bract regulators.

1158 **Table S6:** Genotypes of all RIL and HIF lines used in this study.

1159 **Table S7:** SNP information relative to new KASP genotyping markers used in this study.

1160 **Table S8:** SNP information relative to new sanger genotyping markers used in this study and
1161 associated primers.

1162

1163 **Figure legends**

1164 **Figure 1: The presence of basal bracts is a common, natural trait in *Arabidopsis thaliana***
1165 **with quantitative variations among genetic backgrounds. A, Examples of bracts (red**

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1166 arrows) in different angiosperms. *Brassicaceae* are mostly bractless but some species retain
1167 bracts at the base of inflorescence branches (e.g. in *L. maritima*). In *A. thaliana*, some natural
1168 accessions display basal bracts (e.g. *Tsu-0*) while others do not (e.g. *Col-0*). Schematic
1169 phylogenetic relationships are indicated with a cladogram below the pictures. White stars: first
1170 ebracteate flowers following previous bracteate flowers **B**, (from left to right) *A. thaliana*'s basal
1171 bracts can be true leaves or just small rudimentary filamentous structures at the base of the
1172 floral pedicel (red arrows). These structures are absent in younger flowers, as in the reference
1173 *Col-0* accession (rightmost panel, black arrowhead). **C**, Details of basal bracts by scanning
1174 electron microscopy in a *Tsu-0* inflorescence tip (left panel), showing a bract on the first flower
1175 (red arrow) and swollen base of pedicels on the two following flowers (red arrowhead). Right
1176 panel: pedicels of *Col-0* plants do not show such structures (black arrowhead). **D**, Occurrence of
1177 basal bracts in different accessions, assessed by the percentage of plants with at least one
1178 basal bract in the inflorescence. Each dot is the average value of several plants (number
1179 indicated by dot size) of a scoring assay. A box plot indicates several assays per line and
1180 thicker horizontal black lines are the median value of all scoring assays (range 1-3) in the
1181 accession. *Col-0* and *Tsu* are highlighted in green and red, respectively. The geographical origin
1182 of each strain is located in the world map below. **E**, Definition of a quantitative bract score for a
1183 single plant (see main text for detail). **G**, Quantification of basal bracts in different accessions
1184 using the bract score.

1185

1186 **Figure 2: Flowers bearing bracts express high levels of LFY and do not display mutant**
1187 **phenotypes**

1188 **A, B**. Confocal live imaging of the main meristems of *Col-0* (**A**) and *Tsu-0* (**B**) plants at floral
1189 transition, expressing a pLFY transcriptional reporter (magenta) and a membrane marker
1190 (green) for morphology (top-view projections of stack acquisitions). Green arrowheads:
1191 branches, ordered with decreasing numbers from the floral transition; white arrows: flowers
1192 ordered with increasing numbers from the floral transition. In both genotypes, pLFY expression
1193 is absent from branch meristems and suddenly appears in the first flower onwards.
1194 Representative pictures of at least 6 plants per genotype captured at floral transition.

1195 **C**, side-view of the first flower from image B (*Tsu-0*): a bract (red arrow) is visible on the abaxial
1196 side of this young flower.

1197 **D**, Number of floral organs in flowers with (dark red) or without (light red) bract in *Tsu-0* plants.

1198 **E, F**. Side views by confocal live imaging of *Tsu-0* flowers with bracts (red arrow) expressing the
1199 pLFY reporter (magenta) and the green morphological marker. Flowers show two

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1200 developmental stages older than C and with increasing age from E to F, as shown by the
1201 growing abaxial sepal on top of the flower. pLFY is expressed at low levels in the bract margins
1202 (magenta arrow).

1203 Scale bars: 50 μ m

1204

1205 **Figure 3: Variation in natural basal bract frequency is not correlated with branch position,**
1206 **plastochron length or light conditions, but with variation in flowering time**

1207 **A**, Correlation study between the number of lateral (cauline) branches and the number of bracts
1208 on the main stem only in *Col-0* and *Tsu-0* plants (green and red, respectively). (Pearson test, p-
1209 value > 0.1).

1210 **B**, Effect of different light regimes on bract scores in *Col-0* and *Tsu-0* plants (green and red,
1211 respectively). LD and SD stand for long- and short-day conditions, respectively and CL stands
1212 for continuous light (see methods). Numbers (e.g. 20SD > LD) indicate the number of days in
1213 the first condition before transfer to the second. The number of plants scored is indicated below
1214 each bar.

1215 **C**, Cumulated production of organs over time for *Col-0* and *Tsu-0* plants counted in
1216 microdissected shoot apices (green and red, respectively, each time point averaging 5 to 10
1217 plants): the time window when plants make the floral transition is indicated by a horizontal arrow
1218 and vertical dashed lines. The rate of organ production (inverse to the plastochron) in this period
1219 is computed from the local slope of the curve. Plants were cultured for 21 days in short-day
1220 conditions before transfer in long-day (LD) conditions (see methods).

1221 **D**, Result of three independent experiments of plastochron measurements at flowering transition
1222 (see also fig. S5).

1223 **E**, Differences in flowering time between *Col-0* and *Tsu-0* accession measured as bolting time in
1224 days (left) or as the number of vegetative nodes (rosette leaves and cauline leaves/branches)
1225 before the first flower on the main stem. Plants grown in LD conditions, N = 58 plants per
1226 genotype in two independent replicates.

1227 **F**, Correlation study between the bract score and the flowering time (assayed when the first
1228 flower opens) in different accessions, labelled with different colours. Each dot is a plant and a
1229 linear regression standard deviation is computed for each accession.

1230

1231 **Figure 4: Identification of 4 major QTLs controlling basal bract formation in *Tsu-0***
1232 **suggests unknown genetic pathways. A**, Genetic transmission of bract score in F1 and F2
1233 hybrids from a cross between *Col-0* and *Tsu-0* parent plants. **B**, QTL mapping for bract score in

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1234 a set of RILs identifies four putative QTLs, two in chromosome 1 (1a and 1b) and two in
1235 chromosome 5 (5a and 5b). Dotted horizontal red lines indicate three different significance
1236 thresholds computed from 2000 permutations. On top of the graph, H^2 indicates the (broad-
1237 sense) heritability of the bract score computed among the RIL set. **C**, Genotype of the F6
1238 generation of the line RIL334 in chromosome 1, showing a heterozygotic region overlapping
1239 with QTL1a. **D**, Chromosome 1 genotypes of selected lines forming a heterologous inbred
1240 family (HIF) obtained from RIL334 after two more generations of selfing (F8). **E**, Box plots of
1241 bract scores of the different lines from the “334 family” (blue boxes) with parental controls (*Col-*
1242 *0*: green, *Tsu-0*: red, $N > 20$ plants per line). Segregating bract scores within the family map a
1243 narrower region, QTL1a*, spanning 541 annotated genes. **F**, Genotype of the F6 generation of
1244 the line RIL488 in chromosome 1, showing a heterozygotic region overlapping with QTL1b. **G**,
1245 Chromosome 1 genotypes of selected HIF lines obtained from RIL488 at F8 and (**H**) a box plot
1246 of their bract scores (blue boxes), with parental controls (*Col-0*: green, *Tsu-0*: red, $N > 21$ plants
1247 per line). This maps a narrower region, QTL1b*, spanning 332 annotated genes. In E and H,
1248 lines not sharing the same letter(s) are statistically different (posthoc Tukey analysis with 0.05
1249 sign. level from a glm of bract scores fitted with a quasi-poisson distribution). **I**, Final genetic
1250 mapping of the ‘basal bract’ trait in *Tsu-0* and the location of genes reported to impact bract
1251 development in previous mutant studies.

1252

1253 **Figure 5: Transcriptomic divergence between *Tsu-0* and *Col-0* meristems peaks at the**
1254 **floral transition and suggests an unknown developmental control for natural basal bract**
1255 **formation**

1256 **A**, Scanning electron microscopy showing the evolution of the main meristem in both *Col-0* and
1257 *Tsu-0* at the four different stages (V, L, T, F) used for RNAseq. Plants were first synchronized
1258 by 21 days of a non-inductive short-day light regime before a transfer to inductive long days.
1259 The date after the transfer is indicated in the top-right corner of each picture. Green arrowheads
1260 point to branches (with a leaf) while magenta arrowheads point to the first flowers produced
1261 after floral transition. In *Tsu-0*, red arrows show bracts.

1262 **B**, Dynamics of the expression levels of FT, LFY and AP1 (three important regulators of floral
1263 transition and identity) in both *Col-0* (green) and *Tsu-0* (red) over the four developmental
1264 stages. Differential expression analysis reveals no difference at any stage between the two
1265 accessions.

1266 **C**, Bar plots of the number of genes differentially expressed between two consecutive stages in
1267 *Col-0* (green) and *Tsu-0* (red). The number of changes peaks at the V-to-T stage transition,

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1268 especially in *Tsu-0*.

1269 **D**, Bar plots of the number of differentially expressed genes (DEG) between *Col-0* and *Tsu-0* at
1270 each stage of the time course. T stage is when the highest number of DEG is measured (on top
1271 of the bar, different letters indicate the statistical difference with a chi-square test of
1272 homogeneity after posthoc analysis).

1273 **E**, Dynamics of the expression levels of three previously known “bract” genes, showing a
1274 significant difference at least at the T stage between *Col-0* (green) and *Tsu-0* (red). Two stars
1275 mean that the genes are differentially expressed and the fold change is superior to 1.

1276 **G**, Strategy to cluster genes based on the presence/absence of leaf and/or bract in the different
1277 combinations of stages and genotypes (see text for details).

1278 **H**, Volcano plot of gene expressions at the T stage between the two accessions. All genes
1279 expressed in the SAM are plotted (n=21,652 grey dots) but only the genes fulfilling the
1280 clustering condition defined in G (“putative bract regulators”) are highlighted in orange and blue
1281 for up- and down-regulation, respectively (n=124). Vertical dashed lines: absolute fold change
1282 superior to 1, horizontal dashed line: significance threshold at $5 \cdot 10^{-2}$ (adjusted p.value with fdr
1283 method).

1284 **I**, Representative pictures of micro-dissected meristems in *Col-0* (upper row) and *Tsu-0* (lower
1285 row) just at or before (left) or after (middle) stage T (N>15 for each genotype) and a close-up of
1286 the base of the bolted main stem (right). At stage T, *Tsu-0* meristems display a typical
1287 anthocyanin red coloration just below the meristem, which is not observed in *Col-0*. After
1288 bolting, both genotypes show anthocyanin coloration at the base of the stem. Scale bars: 100
1289 μm (left and middle), 1 cm (right).

1290 **J**, Examples of the expression profile of two candidate genes, showing an up- (left) and a down-
1291 (right) regulation at the T stage. *DFR* is an enzyme involved in anthocyanin biosynthesis while
1292 *FMOGS-OX7* is annotated as a salicylic acid responding enzyme. Two stars mean that the
1293 genes are differentially expressed and the fold change is superior to 1. The bract and bract-less
1294 clusters are outlined with a solid and dashed circle, respectively, while the horizontal dotted line
1295 highlights their separation.

1296

1297 **Figure 6: Massive and complex transcriptional desynchronisation coexist in *Tsu-0* across**
1298 **the floral transition.**

1299 **A**, PCA of RNAseq data from microdissected meristems of *Col-0* and *Tsu-0* over the four
1300 sampled stages (biological replicates averaged per time points). The two main axes of the PCA
1301 can be interpreted as developmental time and genotype, respectively.

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1302 **B**, Examples of temporal registration of gene dynamics (right panels) between *Col-0* (green)
1303 and *Tsu-0* (red) from scaled expression levels (left panel). Dots represent the expression levels
1304 of independent biological replicates, while lines indicate the mean expression level at each time
1305 point. In the right panel, the green (or red) dotted curves represent the fitted models for *Col-0* (or
1306 *Tsu-0*) independently, while the grey dotted curve represents the joint model for both *Col-0* and
1307 *Tsu-0*. If the green and red dotted curves are used, it means the two time series are best
1308 explained by two independent models, indicating they are not similar. Conversely, if the grey
1309 dotted curve is used, it suggests that a single model best explains both time series, indicating
1310 they are similar. The name of the gene plotted is indicated on the left of each row. These four
1311 genes exemplify the four possible categories (from top to bottom, respectively): genes that
1312 cannot be registered (N = 43, e.g. *CYP705A9*), genes with identical temporal dynamics (shift =
1313 0, e.g. *AP1*, see also Fig. 5B) and genes whose expression dynamics in *Tsu-0* must be shifted
1314 negatively (e.g. *DFR*, see also Fig. 5J) or positively (e.g. *AG*) to align with *Col-0*. The last
1315 column provides a biological interpretation of the computed shift (see main text): a null shift
1316 indicates that the expression dynamics in *Tsu-0* stay “in phase” with floral transition while
1317 negative or positive shifts indicate that the expression dynamics in *Tsu-0* are desynchronized
1318 later or earlier, respectively, than the phenotypic progression of floral transition.

1319 **C**, Distribution of heterochronic shifts between *Tsu-0* and *Col-0* on the entire meristematic
1320 transcriptome, computed by the registration method from B. The shift value is colour-coded in a
1321 red-to-blue gradient from -1 to 1.

1322 **D**, GO term enrichment analysis associated with the three categories of heterochronic shifts.
1323 The list of all significant ‘biological process’ GO terms (BH-adjusted p.value < 0.05) was
1324 simplified using semantic similarity (cutoff = 0.7) and the Rich Factor was computed for the
1325 remaining terms, revealing the proportion of genes involved among all the genes associated
1326 with this GO term. Dot size indicates the count of genes and the color scale is the statistical
1327 significance (BH-adjusted p.value) of the enrichment in the shift category. Stars indicate GO
1328 terms referring to developmental processes.