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1 **Ethylene plays a dual role in sex determination and fruit shape in cucurbits.**

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16

17 **Summary**

18 Shapes of vegetables and fruits are the result of adaptive evolution and human selection. Modules
19 controlling organ shape have been identified. However, little is known on signals coordinating
20 organ development and shape. Here we describe the characterization of a melon mutation *rfl*,
21 leading to round fruit. Histological analysis of *rfl* flower and fruits revealed fruit shape is
22 determined at flower stage 8, after sex determination and before flower fertilization. Using
23 positional cloning, we identified the causal gene as the monoecy sex determination gene
24 *CmACS7*, and survey of melon germplasms showed strong association between fruit shape and
25 sexual types. We show that *CmACS7*-mediated ethylene production in carpel primordia enhances
26 cell expansion and represses cell division, leading to elongated fruit. Cell size is known to rise as
27 a result of endoreduplication. At stage 8 and anthesis, we found no variation in ploidy levels
28 between female and hermaphrodite flowers, ruling out endoreduplication as a factor in fruit shape
29 determination. To pinpoint the gene networks controlling elongated versus round fruit phenotype,
30 we analyzed the transcriptomes of laser-capture microdissected carpels of wildtype and *rfl*
31 mutant. This high resolution spatio-temporal gene expression dynamics revealed the implication
32 of two regulatory modules. The first module implicates *E2F-DP* transcription factors, controlling
33 cell elongation versus cell division. The second module implicates *OVATE* and *TRM5*-related
34 proteins, controlling cell division patterns. Our finding highlights the dual role of ethylene in the
35 inhibition of the stamina development and the elongation of ovary and fruit in cucurbits.

36

37 **Introduction**

38 Fruit, the mature ovary of a flower, is a vital structure in the sexual life cycle of angiosperms.
39 Fruit development is initiated by ovule fertilization which promotes the ovary wall to undergo
40 development and differentiation into fleshy or dry fruits. At maturity, fruits enclose and protect
41 seeds, and aid in their dispersal. In angiosperms, fruit initiation and development share
42 evolutionary conserved biological processes but at maturity exhibit an extraordinary diversity in
43 terms of color, size and shape. This diversity is driven by Darwinian natural selection and
44 thousands of years of artificial selection of fruit traits relevant to fruit production and marketing.
45 For instance, fruit attributes such as size and shape are important in fruit harvesting and
46 packaging, with consequences on transportation. Fruit appearance has also a major influence on
47 consumers, preferring fruits of equal weight and uniform shape¹.

48
49 Fruit shape is the result of coordinated spatio-temporal regulation of cell division and expansion.
50 The genetic basis of fruit shape has been assigned to a limited number of genes in several crops²⁻
51 ⁸. In tomato, a model species of fruit shape, five genes have been shown to control fruit shape and
52 development, *FASCIATED (FAS)*, *LOCULE NUMBER (LC)*, *SUN*, *OVATE*, *Ovate-family protein*
53 *(SIOFP20)* and *TONNEAU1 RECRUITING MOTIF 5 (SITRM5)*⁹⁻¹⁴. *LC* and *FAS*, orthologs of
54 *WUSCHEL* and *CLAVATA3* transcription factor, respectively, influence the tomato fruit shape
55 through the regulation of the number of fruit locules^{9,11,15}. *SUN*, a member of the IQ67 Domain
56 (IQD) protein family positively regulates fruit elongation^{12,16,17}. *OVATE*, the founding member of
57 the *OVATE*-family proteins (OFPs), encodes a protein with a conserved ~70 amino acid C-
58 terminal domain, the *OVATE/DUF623* domain¹⁸. Wild type *OVATE* and *SIOFP20* regulate fruit
59 shape through repression of cell division in the longitudinal axis and enhance cell division along

60 the transversal axis^{10,14,19}. SITRM5, a member of the Arabidopsis TRM1-5 clade, interacts with
61 OVATE to regulate cell division in developing ovaries¹⁴.

62
63 The *Cucurbitaceae* is one of the angiosperm families with the most diverse fruits. For instance,
64 fruit size of wild melon (*Cucumis melo* var. *agrestis*) and cucumber (*Cucumis sativus* var.
65 *hardwickii*) are quite small, weighing less than 50 grams²⁰. By contrast, *Atlantic Giant* variety of
66 pumpkin (*Cucurbita maxima*) hold the world record of fruit weight, reaching 1056 kilograms²¹.
67 Fruit shape is also very diverse. Fruits of the *flexuosus* melon varieties can reach more than two
68 meters length with a fruit growth rate of nine centimeters per day²². Fruit shape is also very
69 diverse in bottle gourd and squash (*Cucurbits pepo*) with spherical, cylindrical, elongated,
70 curved, oblate, obovoid, drum-shaped, pear-shaped, spindle-shaped or crooked neck forms^{23,24}.
71 Owing to this extraordinary diversity of fruit shapes and sizes, *Cucurbitaceae* are excellent model
72 systems to understand the molecular mechanisms governing fruit development.

73 Several studies have reported major QTLs controlling cucurbit fruit shapes^{1,2,25-27}. In cucumber,
74 *CsFUL1*, *Short fruit 1* (SF1) and *SF2* encode a FRUITFULL-like MADS-box transcription
75 factor, a cucurbit-specific RING-type E3 ligase and a histone deacetylase complex 1 homologue,
76 respectively²⁸⁻³⁰. *CsFUL1* represses the expression of *CsSUPERMAN* and inhibits auxin transport
77 and thereby regulates cell division and expansion³⁰. In melon, the fruit shape QTL
78 *fsqs8.1/CmFSI8* encodes an OVATE family protein, CmOFP13, orthologous to SIOFP20^{31,32}.

79 Interestingly, some of the cucurbit fruit shape QTLs were shown to be associated with sex
80 determination genes^{2,3,33}. In melon, the fruit shape QTL *fs2.2* co-segregates with the *Monoecy*
81 (separate male and female flowers on the same plant) gene, *CmACS7*, encoding for 1-
82 aminocyclopropane-1-carboxylic acid synthase, the rate-limiting enzyme in ethylene

83 biosynthesis^{1,2,34}. Similarly, in cucumber, the QTL controlling the transition between elongated
84 and round fruit co-segregates with *CmACS7* orthologous gene, *CsACS2*^{3,33,35}. Ethylene is also a
85 key hormone in higher plants commonly associated with fruit ripening^{36,37}. Although genetic
86 associations between *Cucurbitaceae* sex determination genes and fruit development have been
87 proposed, little is known about the link between sex determination, ethylene production and
88 cellular and molecular mechanisms involved in *Cucurbitaceae* fruit shape determination.

89
90 Here, we report the cloning and characterization of a new melon mutant, *round fruit 1 (rf1)*,
91 which encodes a null allele of *CmACS7*. We reveal how ethylene produced by *CmACS7* controls
92 cell division and elongation, providing mechanistic insights into fruit shape regulation in plants.

93

94 **Results**

95 **Elongated versus round fruit shapes are determined before flower anthesis**

96 The melon inbred line Charentais Mono is a monoecious breeding line producing elongated fruit.
97 To identify genes controlling fruit shape, we produced an ethyl methanesulfonate (EMS)
98 mutagenized collection from Charentais Mono line. Phenotyping of 10 000 M2 plants
99 corresponding to 1 000 M2 families led to identification of one mutant with rounded fruits (*rf1*,
100 Figures 1A and 1B). To measure the roundness of *rf1* fruits we calculated the fruit shape index
101 (FSi) as the ratio of the fruit length by the fruit diameter at mature stage. We found fruit length
102 significantly longer in Charentais Mono line (WT) compared to *rf1* mutant. In contrast, fruit
103 diameter did not significantly differ (Figures 1C and 1D), leading to FSi of 1.59 for the WT and
104 1.34 for the *rf1* mutant (Figure 1E). To pinpoint when melon fruit shape is determined, we

105 measured FSi of developing fruits of WT and *rf1* plants, starting from fertilized flower at anthesis
106 stage to mature fruit (Figure S1). We found significant differences of FSi between WT and *rf1*
107 plants, at all investigated fruit developmental stages, suggesting that melon fruit shape is
108 determined before flower fertilization (Figures S1A-S1C). To validate this we measured the
109 ovary shape index (OSi) as the ratio of the ovary length by the ovary width at anthesis stage and
110 before flower fertilization. As for the fruits, we found ovary length significantly longer in WT
111 compared to *rf1* mutant and no significant difference in the width of the ovaries (Figures 1F-1J),
112 leading to OSi of 2.58 for the WT and 1.95 for the *rf1* mutant (Figure 1J). Based on this we
113 concluded that elongated versus round fruit shape is determined during ovary development and
114 before flower fertilization.

115 To investigate the inheritance of the round fruit phenotype, *rf1* plants were back crossed to WT.
116 Consistent with *rf1* being a recessive mutation, F1 hybrid plants developed elongated fruits with
117 FSi similar to WT plants (Figures 1C-1E). Analysis of the F2 population showed 3:1 segregation
118 of elongated versus round fruit phenotypes, consistent with the hypothesis *rf1* is a single-locus
119 recessive mutation leading to round fruit (Table S1).

120

121 ***rf1* locus encodes 1-aminocyclopropane-1-carboxylic acid synthase**

122 To identify *rf1* causal mutation, we sequenced bulked-genomic DNA from M2 plants producing
123 elongated fruits versus round fruits and determined the delta-SNP index. Four SNPs with $\Delta(\text{SNP}$
124 index) superior to 0.5 and mapping to chromosome 2 telomeric region were found linked to *rf1*
125 (Figure 1K). Fine mapping further delimited *rf1* to G1504A transition leading to D279N
126 missense mutation in the sex determination gene *CmACS7* (Figures 1L and 1M). Primary and
127 tertiary protein structure analysis revealed D279N amino acid modification altering a highly

128 conserved protein domain, implicated in the binding of the enzyme cofactor, the pyridoxal 5'-
129 phosphate (PLP)^{38,39} (Figures 1N and S2A-S2B). To investigate whether D279N mutation affects
130 CmACS7 enzymatic activity, we expressed His-tagged recombinant CmACS7 and CmACS7^{D279N}
131 proteins and assayed their activity *in vitro* by monitoring 5'-methylthioadenosine (MTA)
132 formation at different PLP concentrations. The enzymatic assays showed that CmACS7^{D279N}
133 displays very low ACS activity at all PLP concentrations (Figure 1O). These results indicate that
134 CmACS7-mediated ethylene production likely leads to the development of elongated fruits,
135 whereas loss of enzymatic activity leads to round fruits.

136 Previously, we showed that *CmACS7* plays a major role in sex determination and that loss of
137 CmACS7 enzymatic activity leads to the female to hermaphrodite sexual transition⁴⁰. Consistent
138 with this, we found *rfl* plants andromonoecious, developing male and hermaphrodite flowers
139 (Figure S1). To test further the correlation between the development of female flowers and
140 elongated fruit, we phenotyped our previously reported *CmACS7* mutants, *CmACS7*^{G19E},
141 *CmACS7*^{A57V} and *CmACS7*^{D376N}, for fruit shape. CmACS7^{G19E} and CmACS7^{A57V} isoforms
142 showed reduced ACS enzymatic activity whereas CmACS7^{D376N} isoform is mutated in a
143 nonconserved amino acid position predicted to not impair the protein function⁴⁰ (Figure S2C).
144 We found all the mutations leading to female to hermaphrodite flower sexual transition also lead
145 to round fruit development (Figures S1D-S1S). Conversely, the mutation *CmACS7*^{D376N}, not
146 altering sex of the flower do not alter fruit shape.

147 To test if this correlation occurs also in cucumber, we phenotyped five *CsACS2* mutants for fruit
148 development⁴¹. *CsACS2*^{G33C}, *CsACS2*^{P209S} and *CsACS2*^{S399L} isoforms showed reduced to no
149 ACS enzymatic activity whereas *CsACS2*^{S238F} and *CsACS2*^{S249F} isoforms are impaired in
150 nonconserved amino acid positions predicted to not impact the protein function⁴¹. As in melon,

151 *CsACS2* loss of function mutants, *CsACS2^{G33C}*, *CsACS2^{P209S}* and *CsACS2^{S399L}*, developed
152 hermaphrodite flowers and round fruits, whereas mutants not impaired in ACS activity,
153 *CsACS2^{S238F}* and *CsACS2^{S249F}*, developed female flowers and elongated fruits (Figure S3). As the
154 loss of the enzymatic activity of *CmACS7/CsACS2* is associated with round fruit development,
155 ethylene is likely a positive activator of elongated fruit growth. To test this hypothesis, we treated
156 monoecious melon plants with 400 ppm of the ethylene perception inhibitor, silver nitrate, and
157 phenotyped the developed fruits for fruit shape. As expected, treated plants developed
158 hermaphrodite flowers and round fruit (Figure S1T). In summary, these data strongly validate the
159 role of the ethylene produced in the flower by the monoecy genes in the development of
160 elongated fruits.

161

162 ***CmACS7* loss of function allele is associated with round fruit melon accessions.**

163 Previously, we have shown that *CmACS7* has a strong selective sweep signal and has experienced
164 a recent positive selection in andromonoecious accessions⁴⁰. To explore the association between
165 *CmACS7^{A57V}* loss of function allele⁴⁰ and fruit shape, we characterized fruit shape in a panel of
166 190 *Cucumis melo* melon accessions encompassing 15 horticultural groups and 2 sexual types,
167 monoecious and andromonoecious (Figures 2A-2V and Table S2). At least three fruit per
168 accession were measured and the average FSi was calculated. Classification according to
169 *CmACS7* or *CmACS7^{A57V}* genotype showed that accessions carrying functional *CmACS7* allele
170 develop fruit significantly longer than accessions carrying *CmACS7^{A57V}* loss-of-function allele
171 (Figure 2W). This result was consistent with the comparison of female and hermaphrodite
172 flowers in melon and cucumber (Figures S1 and S3) and confirmed the role of functional
173 *CmACS7* in elongated fruit development.

174 We next examined the association of *CmACS7* and *CmACS7^{A57V}* alleles relative to fruit shape
175 index (Figures 2X and 2Y). As observed for the entire panel (Figure 2W), *CmACS7^{A57V}* was
176 strongly enriched in accessions developing round fruit (FSi < 1.5) particularly in the
177 *cantalupensis*, *makuwa* and *reticulatus* horticultural groups (Figure 2Y and Table S2). In
178 contrast, most of the elongated fruit accessions carry the WT *CmACS7* allele and *CmACS7^{A57V}*
179 allele is almost absent in the *flexuosus* horticultural group (Figure 2X and Table S2). In view of
180 the phenotypic association between fruit shape and flower sexual type, and the observed patterns
181 and effects of allelic change in *CmACS7*, we concluded that the presence of round fruit shape in
182 cultivated melon accessions is the consequence of the selection of the andromonoecious
183 phenotype.

184

185

186 **Elongated versus round fruit shapes is determined at stage 8 after flower sex determination**

187 Characterization of *CmACS7/CsACS2* sex transition mutants for fruit shape strongly indicates
188 *Monoecy* gene is pleiotropic, controlling stamen inhibition in female flowers and the development
189 of elongated fruit. To assess whether fruit shape is determined before, during or after sex
190 determination we measured OSi of female and hermaphrodite flower buds at different developing
191 stages (Figure 3). In melon, like in cucumber, female and hermaphrodite flowers at stage 4 are
192 bisexual, developing both stamen and carpel primordia. At stage 6, stamen primordia stop
193 developing in female flowers, leading, at stage 7, to sexually dimorphic female and
194 hermaphrodite flowers⁴². We found no difference in OSi between female and hermaphrodite
195 flower buds until stage 7 (Figures 3A-3E and 3H). At stage 8, we found ovary length
196 significantly longer in WT compared to *rfl* mutant and no significant difference in the width of
197 the ovaries (Figures 3F-3I). Based on this, we concluded that elongated versus round fruit shape

198 is determined during ovary development after sex determination. Consistent with this, *CmACS7*
199 is expressed in carpel primordia of flower buds at stage 4, when female and hermaphrodite buds
200 are not morphologically distinguishable⁴⁰. Quantitative RT-PCR and *in-situ* hybridization show
201 that *CmACS7* is expressed at stage 8 and the accumulation of *CmACS7* mRNA is localized in the
202 central cells of the ovary (Figures 4B and 4C). Further, *CmACS7* expression level and pattern
203 were not different between female and hermaphrodite flowers (Figure 4C), a finding consistent
204 with the fact that *rfl* fruit shape is attributed to the loss of *CmACS7* activity (Figure 1).

205

206 **Developmental and cellular changes leading to fruit shape determination**

207 Organ growth and shape are driven by both cell division and cell growth. These two processes are
208 coordinated but can be independently regulated. To quantify the contribution of the cellular
209 mechanism by which *CmACS7* controls fruit shape, we measured cell numbers and cell sizes
210 along longitudinal and transversal axis of WT female and *rfl* hermaphrodite flowers at stage 8
211 and at anthesis (Figures 4A, 4L and 4M). Although female and hermaphrodite flowers differed
212 significantly in ovary length, they do not differ with regard to cell number in the longitudinal axis
213 (Figure 4A). However, cell length was significantly longer in the WT female flowers. In the
214 transversal axis, we observed significantly more cells with smaller length and width in the *rfl*
215 hermaphrodite flowers. These results suggest that *CmACS7* may regulate fruit length by
216 mediating cell expansion and repressing cell division (Figure 4A).

217 To investigate whether the cell expansion differs uniformly throughout the ovary length,
218 individual cell file along longitudinal axis of female and hermaphrodites flowers was divided into
219 consecutive sectors of 10 cells and measured (Figure 4E). We observed that the average cell
220 length was similar in the top (cells 1 to 30) and bottom ovary ends (cells 110 onwards) (Figures

221 4D, 4F, 4G, 4J and 4K) whereas cells in the sector encompassing the 31st to 110th cells,
222 expressing *CmACS7*, were significantly longer in female compared to hermaphrodite flowers
223 (Figures 4D, 4H and 4I).

224 Endoreduplication is known to be associated with the increase in organ/cell size including
225 fruits^{43,44-46}. To test whether the cell size increase in WT compared to *rfl* is due to
226 endoreduplication, we measured the ploidy level of ovary cells of female and hermaphrodite
227 flowers at stage 8 and at anthesis. We observed for both WT and *rfl* flowers similar ploidy
228 levels, indicating that endoreduplication may not contribute to the observed shape difference
229 (Figures 4N and 4O).

230 Overall, the results demonstrate that *CmACS7*-mediated ethylene biosynthesis positively
231 regulates cell elongation, leading to ovary and thus fruit elongation.

232

233 **Ethylene modulates the expression of cell division and cell elongation promoting genes**

234 At early fruit developmental stages, *CmACS7* mRNA accumulates in the central cells of the ovary
235 (Figure 4B). To obtain an in-depth resolution of the molecular mechanisms controlling ovary
236 shapes, we examined the transcriptome of laser capture microdissected (LCM-seq) ovary cells
237 from the central part of female and hermaphrodite flower buds at stages 4 and 8 (Figures 5A-5C).
238 Pairwise comparisons showed that female flowers at stage 8 (G8) are the main contributor of
239 differentially expressed genes (Figures 5D, S4A and S4B). Overall, we found 2803 upregulated
240 and 2363 downregulated genes in G8 (Data S1).

241 Consistent with the loss of *CmACS7* function explaining the phenotypic variation, we observed
242 many genes involved in ethylene pathway upregulated in G8 (Figure S4E). We grouped the
243 differentially expressed genes by their expression patterns into two clusters, reflecting up- or

244 downregulated genes in the G8 samples (Figures 5E, S4C and S4D). Gene Ontology (GO) term
245 enrichment analysis revealed cluster 1 enriched in GO terms related to response to stimulus,
246 chemical or hormone and cell communication (Figure 5F). In contrast, cluster 2 was enriched in
247 GO terms related to cell division and expansion including translation, DNA metabolic process,
248 cell cycle, cellular process and microtubule-based movement (Figure 5G). Given the role of cell
249 division and expansion in melon fruit shape, we examined the differentially expressed genes and
250 observed that more than two-third of the genes related to cell division (GO:0051301) were
251 downregulated in G8 compared to G4, H4 and H8 samples (Figure S5A), corroborating the
252 reduced number of cells across the ovary width in G8 compared to H8 buds (Figure 4). In
253 contrast, many members of the xyloglucosylate endotransglucosylase/hydrolase (XTH) family,
254 known to govern cell enlargement, were upregulated in G8 compared to H8 buds (Figure S5B).
255 RT-qPCR analysis confirmed the upregulation of *XTH* genes in G8 (Figures 5H-5K).

256 Functional annotation further identified several transcription factors (TFs) of the *E2F-DP* family
257 downregulated in G8 compared to H8 (Figures S5D and S5E). *E2F-DPs* are TFs known to
258 control cell cycle transition in both plants and animals⁴⁷⁻⁴⁹. In *Arabidopsis*, overexpression of
259 *E2F* has been shown to suppress cell elongation and promotes cell division in cotyledons and
260 hypocotyls^{47,50}. Besides, *SWI/SNF-BAF60* TFs, known to inhibit cell elongation in *Arabidopsis*
261 hypocotyl⁵¹, were also found downregulated in G8 compared to H8 (Figure S5E).

262

263 OVATE family proteins (OFPs) have been shown to regulate plant organ size. In tomato, rice and
264 peach, overexpression of *OFP* genes leads to small rounder fruits^{8,10,19}. To regulate cell division
265 and expansion patterns, OFPs interact with Tonneau1 Recruitment Motif (TRMs) proteins¹⁴. To
266 assess the role of *OFPs* and *TRMs* in melon fruit shape, we have identified 21 OFPs and 39

267 TRMs melon proteins orthologous to Arabidopsis and tomato OFPs and TRMs proteins (Figures
268 S6A and S6B). Current transcriptomic analysis and RT-qPCR revealed that among the
269 differentially expressed genes, *OFPs* gene expression was mainly downregulated in G8 compared
270 to H8 samples (Figures 5L-5O). In contrast, *TRMs* were mainly upregulated in G8 compared to
271 H8 (Figures 5P-5R).

272

273

274 **Discussion**

275 The plant hormone ethylene plays a key role in development, senescence and adaptation to biotic
276 and abiotic stresses^{52,53}. In cucurbits, flower and fruit development can be divided into five major
277 phases: the initiation of floral organs, the sexual dimorphism, the flower anthesis, the fruit
278 development and the fruit maturation. Ethylene intervenes as positive and negative regulator
279 through all these developmental phases⁵⁴. Specifically for the flower developmental phase, we
280 previously showed that ethylene is the key hormonal switch controlling sexual organs
281 development^{40,41,55,56}. Here we demonstrate that ethylene controls fruit shape after the sex
282 determination and before the flower anthesis phase.

283

284 Organ shapes (e.g. fruit shape) is the result of coordinated spatial-temporal cell division and
285 expansion. We show that CmACS7/CsACS2-mediated ethylene production is necessary for both
286 the development of elongated fruits and female flower development (Figures 1, S1 and S3),
287 consistent with the mapping of the fruit shape QTL *FS2.2* at vicinity of the *Monoecy* locus
288 controlling sex determination in melon².

289 In melon, genetic diversity analysis has shown that *CmACS7* allele leading to andromonoecy is
290 under recent positive selection⁴⁰ to either permit flexibility in resource allocation to male and
291 female function⁵⁷⁻⁶⁰, or for elevating male function, to increase pollen donation⁶¹. Besides flower
292 sex determination, fruit shape is another important domestication trait in vegetable and fruit
293 crops. To analyze the relation between fruit shape and sex determination, we investigated 190
294 melon accessions that display diverse fruit shapes and sexual types (Figure 2). We found that
295 monoecious accessions develop fruits longer than those of andromonoecious accessions (Figure
296 2W). Previously, we have demonstrated that *CmACS7*^{A57V} allele is monophyletic and under recent
297 positive selection in andromonoecious melon accessions⁴⁰. Here, we also show that *CmACS7*^{A57V}
298 allele prevails in melon accessions developing round fruits (Figure 2Y). These data further
299 demonstrate that *CmACS7*-mediated fruit shape and sexual determination has been co-selected
300 during melon domestication and further spread by continuous selection of new varieties
301 worldwide. It is important to highlight that even though most melon accessions harboring the
302 *CmACS7*^{A57V} allele develop round fruits, we found some accessions with the *CmACS7*^{A57V} allele
303 developing elongated fruits (Figure 2X). To correct the round shape melon breeders have
304 screened melon germplasms and identified QTLs that modify fruit shape^{1,27,62}. Recently, the
305 melon fruit shape QTL *fsqs8.1/CmFSI8* was shown to encode the OVATE family protein
306 CmOFP13, orthologous to AtOFP1 and SlOFP20^{31,32}. Variations at the *CmOFP13* locus could
307 explain the round fruit phenotype observed in melon accessions harboring the *CmACS7* allele
308 (Figure 2Y).

309
310 *CmACS7/CsACS2* expression inhibits stamen development in a non-cell-autonomous way^{40,41}. In
311 contrast, *in situ* expression analysis showed *CmACS7* expression coincides with the cell

312 enlargement zone in the central region of developing ovaries, pointing toward cell-autonomous
313 control of fruit shape (Figure 4). LCM-seq analysis identified two clusters of gene expression
314 patterns, corresponding to genes up or down regulated in G8, respectively. Consistent with the
315 histological analysis pointing toward inhibition of cell division and enhanced cell elongation as
316 the cause of elongated fruit shape, we found down regulation of *E2F-DPs* and *SWI/SNF-BAF60*
317 TFs known to suppress cell elongation and promote cell division^{47,50,51}.

318 Regulation of microtubule orientation is an important step in cellular regulation of organ shape⁶³⁻
319 ⁶⁶. We found *OFPs* genes downregulated and *TRMs* genes upregulated in female compared to
320 hermaphrodite flower buds (Figure 5). TRMs proteins interact with TONNEAU1 (TON1) and
321 Protein Phosphatase2A (PP2A) to target the TTP (TON1–TRM–PP2A) complex to cortical
322 microtubules to delineate the location of the cell division plane⁶⁷⁻⁶⁹. The contrasting expression of
323 *OFPs* and *TRMs* genes corroborate with the cell size and number differences observed in the fruit
324 shape controlling zone of the ovary. Consistent with this, overexpression of *OFPs* or loss-of-
325 function mutations in *TRMs* resulted in rounder fruits^{8,14,19,67} and QTLs controlling fruit shape
326 were found mapping at the vicinity of *OFPs* or *TRMs* genes^{14,70,71}. In Arabidopsis,
327 overexpression of *AtTRM1* and *AtTRM2* resulted in elongated organs⁷². GO enrichment analysis
328 also revealed major changes in genes related to microtubule-based movement (Figure 5G). The
329 process of cell division and expansion requires dynamic spatial reorganization of microtubule
330 network. Kinesins are a superfamily of microtubule-dependent motor proteins and they play
331 critical roles in various processes such as cell division and alteration of cell morphology⁷³.
332 Surprisingly, transcripts for majority of the differentially expressed kinesin proteins were found
333 to be downregulated in G8 compared to H8 (Figure S5C). It would be interesting to study the role
334 of kinesins in governing the fruit length, if any. XTH enzymes are a large family of cell wall-

335 modifying enzymes that play a central role in the cell wall expansion and re-modelling. We found
336 a large number of XTH enzymes upregulated in cells undergoing enlargement. These protein
337 family were also found to be induced by ethylene in rose, arrowhead tubers and persimmon⁷⁴⁻⁷⁶,
338 pointing toward a general mechanism controlling expression of XTH enzymes by ethylene.

339
340 Treatment of melon plant with ethylene perception inhibitor, silver nitrate led to transition from
341 elongated to round ovary and fruit development. In *Ranunculus sceleratus*, ethylene treatment
342 has been shown to promote petiole elongation growth⁷⁷. These two experiments highlight the
343 dual role of ethylene as an inhibitory-hormone for cell division rate and a promoting-hormone for
344 cell enlargement. Recently, fine-tuning of ethylene homeostasis through a RING-type E3 ligase
345 was also reported to control cucumber fruit elongation, suggesting conserved mechanisms
346 controlling organ shape in plants²⁸.

347
348 In summary we propose a model in which *CmACS7* orchestrate sex determination and flower and
349 fruit shape. Expression of *CmACS7* inhibits stamen development in non-cell-autonomous manner
350 and promotes cell elongation in the carpel to lead to elongated fruit, in a cell-autonomous
351 manner. This process includes activation of cell elongation and inhibition of cell division
352 mechanisms. At the molecular level ethylene produced locally by *CmACS7* lead to repression of
353 cell division promoting genes and upregulation of cell elongation promoting genes. These
354 processes occur after the flower acquire their sexual identity and before flower anthesis (Figure
355 6).

356
357

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368

369 **Author contributions**

370 C.T. and S.F. contributed to the generation of the mutant lines; C.C. contributed to the laser
371 capture microdissection; A.Bo., S.B. and R.D performed bioinformatic analyses and contributed
372 to the gene expression analysis; S.B. and H.M. performed the cell biology and histological
373 experiments; N.G., C.D., S.F. A.Bo. carried out the plant phenotyping of melon lines and
374 accessions; A.Bo., C.D. and A.Be. conceived and designed the study, supervised the work,
375 analyzed the data and wrote the manuscript.

376

377 **Declaration of Interests**

378 The authors declare no competing interests.

379

380

381 **Figure legends**

382 **Figure 1. *rf1* develops round fruit and encodes *CmACS7*.**

383 (A,B) Elongated (A) and round (B) fruit developed on WT and *rf1* mutant plant, respectively.

384 Bars= 5 cm. (C-E) Boxplots of fruit length (C), fruit diameter (D) and fruit shape index (E) in the

385 WT, *rf1* and F1 plants (n=10). (F,G) Flowers of WT (F) and *rf1* (G) plants at anthesis. Bars= 1

386 cm. (H-J) Boxplots of ovary length (H), ovary width (I) and ovary shape index (J) in the WT, *rf1*

387 and F1 plants (n=20). Data in (C-E) and (H-J) are displayed as box plot whiskers representing

388 $\pm 1.5 \times$ the interquartile range; horizontal lines, medians. ***, $P < 0.001$ (two-tailed Student's *t*-

389 test). (K-M) Cloning of the *rf1* locus. (K) The delta SNP index (Δ SNP index) between elongated

390 and round bulks. (L) Schematic of the mapping region of the *rf1* locus. The green and blue

391 arrows represent annotated genes. Orange dashed lines indicate the physical position of the

392 induced EMS mutations. (M) Structure of the *CmACS7* gene and position of the missense

393 induced mutation D279N. Green boxes and black lines represent exons and introns, respectively.

394 (N) Amino acid alignments of *CmACS7* with homologous proteins from *Cucumis sativus* (*Cs*),

395 *Vitis vinifera* (*Vv*), *Cucurbita maxima* (*Cmax*), *Arabidopsis thaliana* (*At*), *Solanum lycopersicum*

396 (*Sl*), *Petunia hybrida* (*Ph*), *Medicago truncatula* (*Mt*), *Momordica charantia* (*Mc*), *Triticum*

397 *aestivum* (*Ta*) and *Picea glauca* (*Pg*). Numbers above the alignment indicate the amino acid

398 positions along the *CmACS7* protein. Box 5 indicates a conserved domain in ACS proteins. (O)

399 Effect of PLP concentration on the ACS enzymatic activity of *CmACS7* (blue bars) and D279N

400 (orange bars) protein forms. See also Figures S1-S3 and Table S1.

401

402

403 **Figure 2: Round fruit phenotype is associated with *CmACS7^{A57V}* allele.**

404 (A-V) Morphological variation of melon fruits from the diversity panel (n=190) representing 15
405 horticultural groups. (A,B) *acidulus*, (C) *agrestis*, (D) *ameri*, (E) *chandalak*, (F) *chate*, (G)
406 *chinensis*, (H) *chito*, (I) *dudaim*, (J,K) *flexuosus*, (L-N) *inodorus*, (O) *cantalupensis*, (P)
407 *conomon*, (Q-T) *makuwa*, (U) *momordica*, (V) *reticulatus*. The picture shows a compilation of
408 fruits from the diversity panel with different colors, shapes, and sizes to illustrate the phenotypic
409 variation present of the fruit shape in melon. For more details please see Table S2. Bar=10 cm.
410 (W) Boxplots of fruit shape index. Data are displayed as box plot whiskers representing $\pm 1.5 \times$
411 the interquartile range; horizontal lines, medians. $p=5.1e-4$ (two-tailed Student's *t*-test). (X,Y)
412 Allele frequencies of *CmACS7* and *CmACS7^{A57V}* in melon accessions developing elongated fruits
413 (n=74) (X) and round fruits (n=116) (Y). See also Table S2.

414

415 **Figure 3: Fruit shape is determined after sex determination,**

416 (A,B) Confocal images of WT (A) and *rf1* (B) flowers at stage 5 before sexual dimorphism. St,
417 stamen; C, carpel. Bars= 100 μ m. (C-E) Carpel length (C), carpel cell number (D) and average
418 cell length (E) in the longitudinal axis of WT and *rf1* flowers at stage 5. Values are means \pm s.d.
419 derived from 3 flowers. (F,G) Confocal images of WT (F) and *rf1* (G) flowers at stage 8 after
420 sexual determination. Bars= 500 μ m. (H-I) Ovary length (H) and width (I) of WT and *rf1*
421 flowers at different developmental stages (n=9). Data in (H) and (I) are displayed as box plot

422 whiskers representing $\pm 1.5\times$ the interquartile range; horizontal lines, medians. ***, $P < 0.001$
423 (two-tailed Student's *t*-test).

424

425 **Figure 4: Cell number and cell length of ovaries from WT and *rf1* flowers.**

426 (A) Characteristics of ovaries from WT and *rf1* flowers. Values are means \pm s.d. derived from 7
427 flowers.. (B) *CmACS7* *in situ* expression at flower developmental stage 8. Bar= 250 μ m. (C)
428 Quantitative real-time PCR of *CmACS7* in WT and *rf1* flowers at stage 8. Values are means \pm s.d.
429 of three biological replicates. (D) Cell length averaged from consecutive sectors of 10 cells along
430 the longitudinal axis of the ovary. Values are means \pm s.d. derived from 5 flowers. (E)
431 Longitudinal cell layers of WT melon flower at stage 8. Bar= 10 μ m. (F-K) Magnification of the
432 WT (F,H,J) and *rf1* (G,I,K) flower cross-section in the apical (F,G), median (H,I) and basal
433 (J,K) ovary regions. Bars= 25 μ m. (L) Boxplots of Cell length and (M) cell width at flower
434 anthesis (n=10). Data in (L,M) are displayed as box plot whiskers representing $\pm 1.5\times$ the
435 interquartile range; horizontal lines, medians. (N,O) Ploidy analysis by flow cytometry in WT
436 and *rf1* flowers at stage 8 (N) and at anthesis (O). Values are means \pm s.d. of five biological
437 replicates. Statistical *p* values are calculated using two-tailed Student's *t*-test. n.s: no statistically
438 significant difference. See also Table S3.

439

440 **Figure 5: Gene expression profiling of female and hermaphrodite flowers by LCM-Seq.**

441 (A-C) Laser capture microdissection of the ovary median region expressing *CmACS7*. (D)
442 Differentially expressed genes in pairwise comparison groups. (E-G) Gene-wise hierarchical

443 clustering heat map (E) of all 5,166 differentially expressed genes (adjusted P value < 0.001)
444 showing segregation into two clusters. The z -score scale represents mean-subtracted regularized
445 log-transformed read counts. (F) Cluster 1 ($n = 2,803$) includes genes with increased expression
446 in female flowers at stage 8 (G8). (G) Cluster 2 ($n = 2,363$) includes genes downregulated in G8.
447 Data in (F) and (G) are displayed as box plot whiskers representing $\pm 1.5\times$ the interquartile
448 range; horizontal lines, medians. Enriched GO terms are shown to the right. (H-R) Quantitative
449 real-time PCR of cell expansion (H-K), *OFPs* (L-O) and *TRMs* (H-J) genes in female and
450 hermaphrodite flowers. Values are means \pm s.d. of three biological replicates. *, $P < 0.05$; **, $P <$
451 0.01 ***, $P < 0.001$ (two-tailed Student's t -test). H4, hermaphrodite flower at stage 4; G4: female
452 flower at stage 4; H8, hermaphrodite flower at stage 8; G8: female flower at stage 8. See also
453 Figures S4-S6, Table S3 and Data S1.

454

455 **Figure 6. Proposed model for *CmACS7*-dependent regulation of melon fruit shape.**

456 At developmental stage 5, female and hermaphrodite carpel-bearing flowers express functional or
457 non-functional *CmACS7* protein isoform in the carpel, respectively. Functional *CmACS7*
458 isoform produces ethylene repressing the development of stamens and leading to the
459 development of female flowers. After sexual differentiation, at stage 8, *CmACS7* continues to be
460 expressed and to produce ethylene in the developing carpels. Ethylene downregulates the
461 expression of *OFPs*, *E2F-DPs* and *SWI/SNF-BAF60* genes and induces the expression of *TRMs*
462 and *XTHs* genes leading to cell elongation and the development of elongated fruit. Expression of
463 the nonfunctional *CmACS7*^{D279N} isoform results in the expression of *OFPs*, *E2F-DPs* and

464 *SWI/SNF-BAF60* genes and downregulation of *TRMs* and *XTHs* genes leading to induced cell
465 division, reduced cell elongation and finally to the development of round fruit.

466

467

468 **STAR Methods**

469 **RESOURCE AVAILABILITY**

470 **Lead contact**

471 Further information and requests for resources and reagents should be directed to and will be
472 fulfilled by the Lead Contact, Abdelhafid Bendahmane (abdelhafid.bendahmane@inrae.fr).

473

474 **Materials availability**

475 This study did not generate new unique reagents.

476

477 **Data and code availability**

478 Accession numbers are listed in the key resources table. This paper does not report original code.
479 The high-throughput sequencing datasets generated in this study have been deposited in the
480 Sequence Read Archive (SRA) under the accession number PRJNA814521. Other data
481 supporting our findings are available in the manuscript file or from the corresponding author
482 upon request.

483

484

485 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

486 **Plant Material**

487 The seeds of Charentais Mono line of melon (*Cucumis melo* L.) were treated with ethyl
488 methanesulfonate (EMS) as described in Dahmani-Mardas et al.⁷⁸. The M1 plants were self-
489 pollinated, and the round fruit line *rfl* was identified in the M2 population. Then, *rfl* was crossed
490 with the Charentais Mono line and an F2 population was derived from self-crossed F1 plants.
491 Mutant melon lines harboring either of the CmACS7^{D376N}, CmACS7^{G19E} and CmACS7^{A57V} were
492 used in this study⁴⁰. Similarly, cucumber wild-type and mutant lines harboring either of the
493 CsACS2^{G33C}, CsACS2^{S238F}, CsACS2^{S249F}, CsACS2^{P209S} and CsACS2^{S399L} were also used in this
494 study^{41,79}. Plants were grown in the greenhouse in the spring and summer under standard
495 agronomic conditions and evaluated for flower sex type and fruit shape during three consecutive
496 growing seasons. For the 190 melon accessions of the diversity panel, encompassing 15
497 horticultural groups and monoecious and andromonoecious sexual types, were grown at INRAE
498 GAFL Avignon station. 2 plants for each accession were phenotyped and 3 fruits per plant were
499 scored for mature fruit length, diameter and shape index. Representative fruits from each
500 accession are shown in Figures 2A-2V.

501

502 **METHOD DETAILS**

503 **Measurement of cell number and cell size**

504 To measure the ovary cell size and number, fresh-picked flower buds from Charentais Mono and
505 *rfl* plants were sampled at different developmental stages 4, 7, 8 and 9 and fixed in FAE solution
506 (formaldehyde: acetic acid: 70% ethanol; 1:1:18 ration) for 24 hours and then washed four times

507 with 70% ethanol. For the measurements, flower buds were stained with 0.1 mM propidium
508 iodide (Sigma-Aldrich) for 1 hour and longitudinal images were recorded using the Zeiss LSM
509 880 confocal microscope. The cell size and number were then calculated using Image J software.
510 The means and standard errors were calculated from the measurements of at least 5 flower buds
511 for each genotype. Student *t*-test was used to compare the cell number and cell size difference
512 between the WT and *rf1* plants.

513

514 **BSA-Seq Mapping Approach**

515 To map the *rf1* causal mutation, an F2 population was constructed by crossing *rf1* mutant plant
516 with WT Charentais Mono plant. More than 200 F2 plants were phenotyped and sampled for
517 individual genomic DNA extraction. Mutant and WT pools were created by mixing equal ratio of
518 genomic DNA from round-fruit and elongated-fruit F2 plants. Genomic DNA pools were shared
519 for preparation of sequencing libraries following the recommendation of Illumina TruSeq DNA
520 PCR-free prep kit. Reads of the two bulks (mutant and WT) were aligned to the reference melon
521 genome⁸⁰. For each bulk, the SNP-index across all loci was calculated as the proportion of reads
522 that were different from the reference allele. The delta (Δ) SNP-index was calculated by
523 subtracting the SNP-indices of the two bulks at each locus (SNP-index_mutant – SNP-
524 index_WT).

525

526 **Expression, purification and enzymatic activity assays of recombinant protein**

527 Recombinant proteins, CmACS7 and CmACS7^{D279N}, were expressed, purified and assayed as
528 described in Boualem et al.⁴⁰. Briefly, *CmACS7* cDNAs from monoecious, and *CmACS7*^{D279N}
529 TILLING mutant were cloned in pET-15b vector as His6-tagged proteins and expressed in *E. coli*

530 BL21(DE3)pLysS cells. Protein expression was induced by adding IPTG (0.5 mM) and cells
531 were grown for 5 hours at 25°C. Cells were harvested and disrupted on ice by sonication in the
532 lysis buffer using five pulses of 30 seconds at 20 kHz with 3 minutes cooling on ice between each
533 pulse. The supernatant separated from cell debris was applied to a Ni-IDA 15 ml column (Sigma,
534 France). Wild type (CmACS7) and mutant (CmACS7^{D279N}) forms of protein were then eluted
535 with the same buffer. CmACS7 and CmACS7^{D279N} enzyme activity was determined by
536 monitoring the MTA formation. Specific activity measurement was performed on 3 different
537 enzyme preparations. Specific activities were measured on dialyzed enzymes in the presence of
538 60µM SAM and various PLP concentrations. Residual activities (%) corresponds to the specific
539 activity measured / specific activity measured for CmACS7 enzyme at 300µM PLP.

540

541

542 **Protein structure modeling**

543 The CmACS7 three-dimensional structures were generated using the Geno3D server
544 (<http://geno3d-pbil.ibcp.fr>). Superposition of the tomato ACS structure (1IAY.pdb) determined
545 by x-ray crystallography (81) and the CmACS7 model was carried out and visualized using the
546 Chimera server (<http://www.cgl.ucsf.edu/chimera>).

547

548 ***In situ* hybridization**

549 CmACS7 in situ hybridization was performed as described in Boualem et al.⁴⁰. Primers used for
550 this experiment are listed in Table S3.

551

552 **Identification of OFP and TRM proteins in melon.**

553 To identify candidate OFP and TRM proteins, the melon database
554 (<http://www.cucurbitgenomics.org/>) was searched first using the keywords ‘OFP’ or ‘TRM’. In
555 addition, Arabidopsis and tomato OFP and TRM protein sequences were downloaded from The
556 Arabidopsis Information Resource (<http://www.arabidopsis.org/>) and the Sol Genomics Network
557 (<http://solgenomics.net/>), respectively. These sequences were used to identify homologous
558 peptides from melon by performing a BLASTP search at melon genome v3.5 database
559 (<http://cucurbitgenomics.org/>). The BLAST E-value was set to 1e⁻³. Finally, repeated and
560 incomplete sequences were removed manually and the non-redundant CmOFP and CmTRM
561 sequences were subjected to further analyses.

562

563 **Sequence and phylogeny analysis**

564 Multiple sequence alignment of full-length protein sequences of CmACS7 with homologous
565 proteins from *Cucumis sativus* (*Cs*), *Vitis vinifera* (*Vv*), *Cucurbita maxima* (*Cmax*), *Arabidopsis*
566 *thaliana* (*At*), *Solanum lycopersicum* (*Sl*), *Petunia hybrida* (*Ph*), *Medicago truncatula* (*Mt*),
567 *Momordica charantia* (*Mc*), *Triticum aestivum* (*Ta*) and *Picea glauca* (*Pg*) was performed using
568 the ClustalW (<http://www.ebi.ac.uk/Tools/clustalw2>). Phylogenetic trees were constructed by
569 MEGAX (<http://www.megasoftware.net/index.html>) based on the Neighbor-Joining method.

570

571 **Silver nitrate treatment**

572 To assess the effect of AgNO₃ on fruit shape, 400 ppm of the ethylene perception inhibitor,
573 AgNO₃ solution was periodically sprayed on the leaves of WT Charentais Mono line at 20
574 internodes developmental stage. About 3 weeks after treatment, perfect flowers appeared and

575 were selfed. The fruits developed from AgNO₃-induced hermaphrodite flowers were phenotyped
576 for fruit shape index.

577

578

579 **Flow Cytometry and ploidy analysis**

580 To determine the flower ploidy level, young flower buds at stage 8 was chopped with a new razor
581 blade in 1ml of nuclei-isolation buffer⁸². Suspended nuclei were filtered through a 40- μ m Fisher
582 brand cell strainer, treated with RNase (5U/mL), and stained with propidium iodide (0.001
583 μ g/100 μ L sample). Ploidy level of ~10.000 nuclei was determined using a Cyflow SL3 flow
584 cytometer (Partec-Sysmex). Ploidy histograms were quantitatively analyzed with DPAC software
585 (Partec).

586

587 **Transcriptome sequencing from laser capture microdissected tissue**

588 Transcriptomic analysis was carried out on laser capture microdissected central region of carpels
589 for both female and hermaphrodite flowers from the WT Charentais Mono and *rfl* plants,
590 respectively. Two different developmental stages (stages 4 and 8) were chosen such that the
591 transcriptomic profiles can be compared before and after the divergence in ovary shape index
592 (OSi) between the female and hermaphrodite flowers. Female and hermaphrodite flower buds at
593 stage 4 were referred to as G4 and H4, respectively. Female and hermaphrodite flower buds at
594 stage 8 were referred to as G8 and H8, respectively.

595 *Tissue embedding*

596 Flowers buds were fixed in RCL2 (Excilone) with 0.01% triton. For tissue fixation, samples were
597 placed under vacuum 4 times for 15 min and kept in the fixative overnight at 4 °C. Samples were
598 then dehydrated at 4 °C in a graded series of ethanol (70% for 30 min, 96% for 30 min, 100% for
599 3 × 30 min), followed by a graded series of ethanol:histoclear bath (3:1, 1:1, 1:3 for 1 h each).
600 HistoClear was then substituted by Surgipath Paraplast Plus tissue embedding media (Leica
601 Biosystems) and incubated overnight at 60 °C. Finally, flowers were embedded into paraffin
602 blocks, cooled and stored at −20 °C.

603 *Laser capture microdissection*

604 A Rotary microtome (HM 3555 Microtom) was used to cut 8 µm thick longitudinal sections of
605 the embedded flowers. Ribbon of flower sections were stretched on UV-treated, 1 mm PEN-
606 membrane covered slides (Arcturus Bioscience, Excilone) such that each slide corresponds to 15–
607 25 sections of flowers. Slides were deparaffinized, and laser capture microdissection was
608 immediately conducted on a Palm DIC FLUO Microdissection System (Zeiss). The contours of
609 central carpel sector encompassing the 31st to 110th cells were cut with the laser and target
610 regions were catapulted into Adhesive cap 500 clear (Zeiss).

611 *RNA extraction*

612 Immediately after dissection, cells were lysed using the PicoPure® RNA Isolation Kit (Arcturus
613 Bioscience, Excilone) and stored at −20 °C before RNA extraction. RNA quality and
614 concentration were evaluated with a Bioanalyser 2100 (Agilent Technologies) on Agilent RNA
615 Pico chips. RNA recovery ranged from 900–2000 pg/µl, with a RIN above 7.

616 *Preparation of the sequencing libraries*

617 2 ng of total RNA was used for each cDNA library preparation using the SMARTer Ultra Low
618 RNA Kit for Illumina Sequencing from Clontech according to manufacturer's instructions.

619 Libraries were sequenced on the HiSeq2000 platform Illumina® and 30 to 50 million paired-end
620 reads per sample were obtained. Read quality was assessed using FastQC (version 0.11) and
621 STAR (version 2.7) has been used to generate the mapping files. The mapped reads were
622 assigned to genes with featureCount (v2.0.0). DESeq2 (version 1.30.1) was used to identify the
623 differentially expressed (DE) genes (adjusted p value <0.001 and $\log_2(\text{FC}) > 1$ or <-1).

624

625 **Hierarchical clustering and GO analysis**

626 The DE genes were subjected to hierarchical clustering analysis using Perseus to identify the co-
627 expressed DE genes (83) GO enrichment analysis was performed on AgriGOv2 web server⁸⁴.

628

629 **Reverse transcription polymerase chain reaction (RT-PCR) and qPCR**

630 For the RT-qPCR, the total RNAs from laser capture microdissection experiments were used to
631 validate the gene expression pattern. Primer design was performed with the Primer3 software
632 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Primers sequences are listed in
633 Table S3. To check specificity of the designed primers, all amplicons were sequenced and blasted
634 against NCBI database. Polymerase chain reactions were performed in an optical 384-well plate
635 with the Bio-Rad CFX96 Real-time PCR apparatus, with qPCR MasterMix Plus for SYBR®
636 Green I w/o ROX (Eurogentec) and according to manufacturer's instructions. PCR amplification
637 specificity was verified by a dissociation curve (55 °C to 95 °C). A negative control without
638 cDNA, technical replicates on three independent synthesis of cDNA (derived from the same
639 RNA sample), and three independent biological experiments were performed in all cases. Gene
640 expression is normalized to the expression levels of housekeeping genes: *CmActin2* and *CmADP*

641 (primers shown in Table S3). The gene relative expressions were determined as described in
642 Eleblu et al.⁸⁵.

643

644 **Genotyping**

645 The genotyping of 81 melons accessions for the C to T nucleotide transition leading to A57V
646 amino acid substitution was carried out with the cleaved amplified polymorphic sequence
647 (CAPS) marker described in Boualem et al.⁴⁰ and on the basis of *Alu1* restriction site
648 polymorphism. Briefly, melon genomic DNA was extracted from young leaves following CTAB
649 method and was used as matrix for *CmACS7* PCR amplification using primers described in Table
650 S3. PCR products were then digested by *Alu I* restriction enzyme to assess the presence absence
651 of the C to T nucleotide transition.

652

653 **QUANTIFICATION AND STATISTICAL ANALYSIS**

654 For the fruit and ovary shape phenotypic analysis in WT Charentais Mono and *rf1* plants, 10
655 fruits from at least 5 independent plants for each genotype were analyzed. For the ovary shape
656 phenotypic analysis, 20 flowers at anthesis from at least 5 independent plants for each genotype
657 were analyzed.

658 Statistical significance analysis between two groups was performed using the Student's *t*-test.

659 For RNA-seq analysis, pool of laser captured microdissected samples from 3 flower buds has
660 been used as one biological replicate. Three biological replicates were performed for RNA-seq.

661 For RT-qPCR analysis, the expression values correspond to the average of three biological
662 replicates, with three technical replicates.

663

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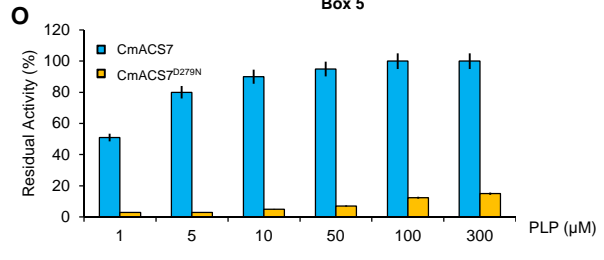
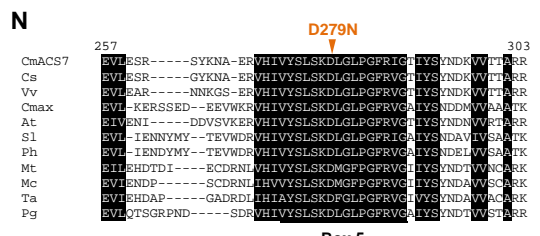
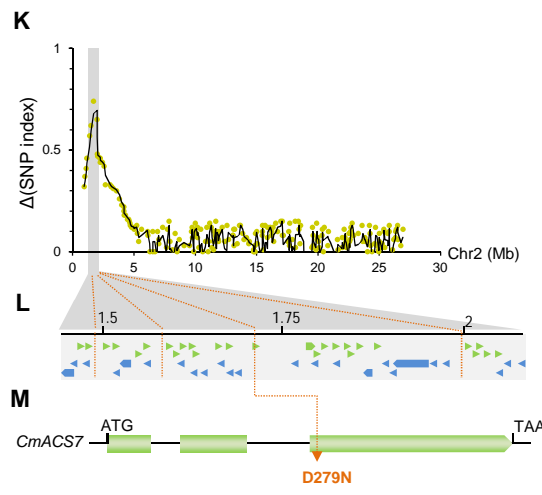
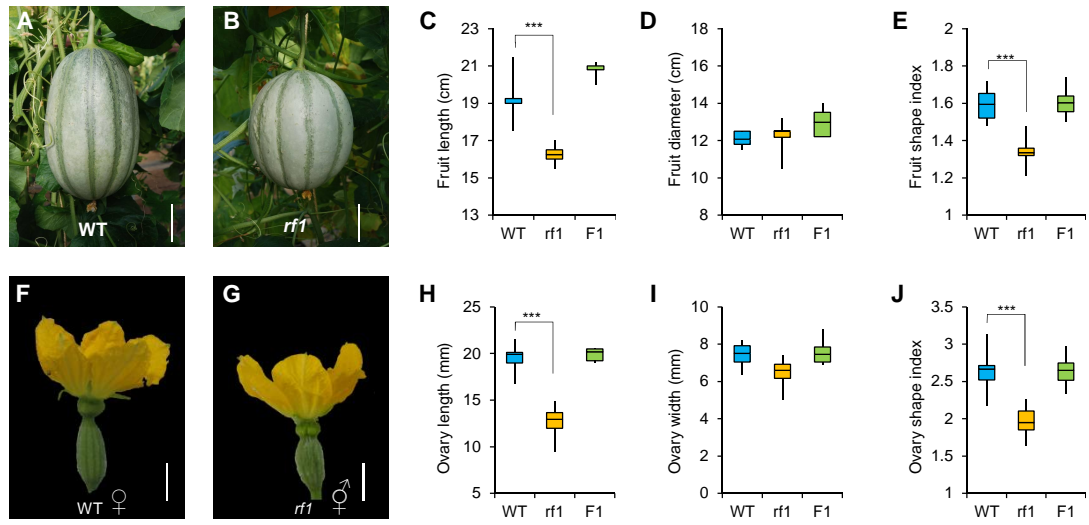
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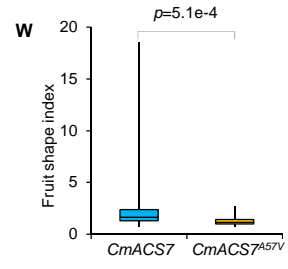
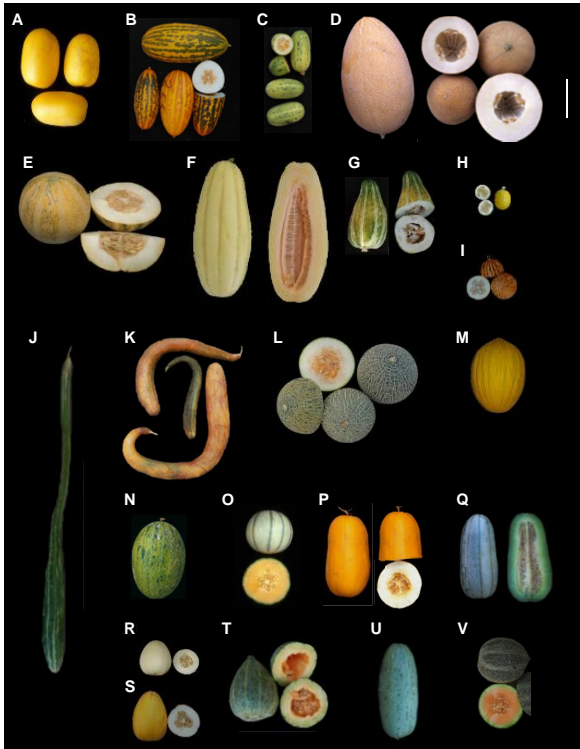
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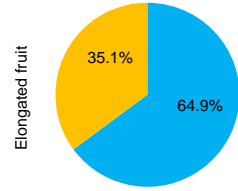
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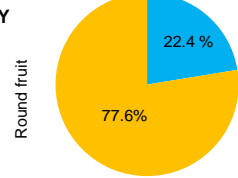


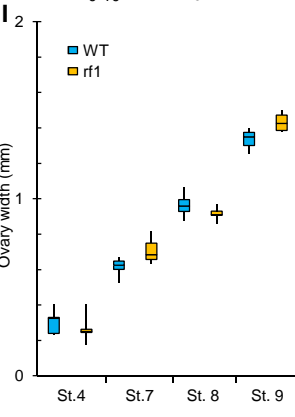
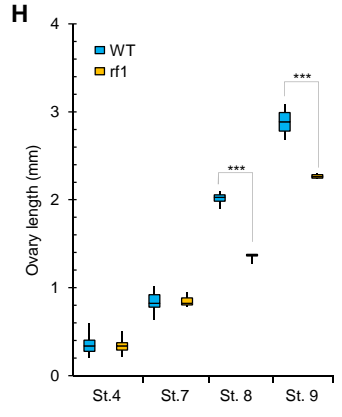
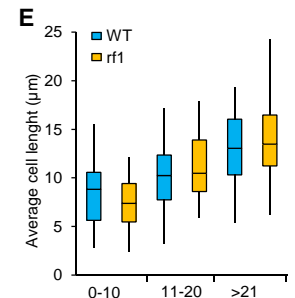
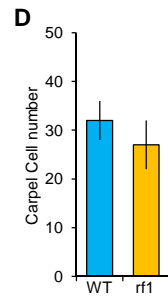
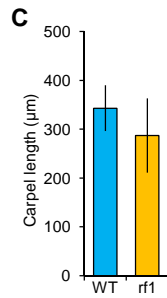
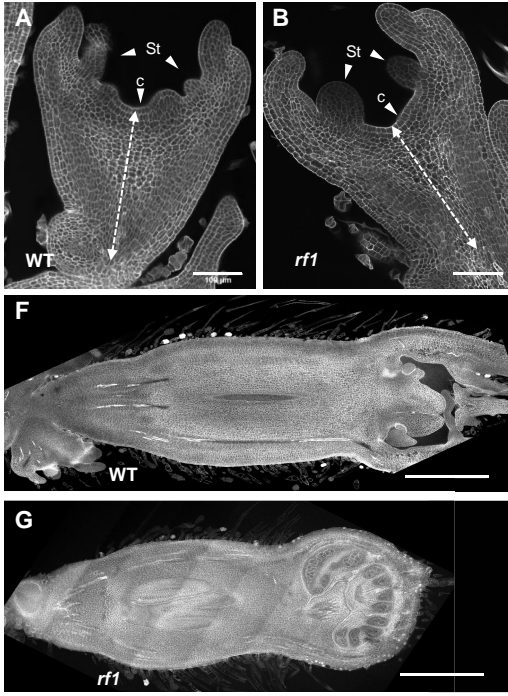
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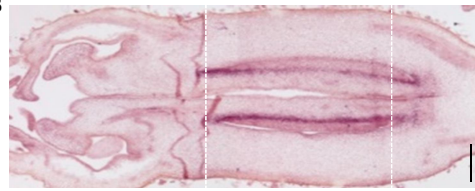




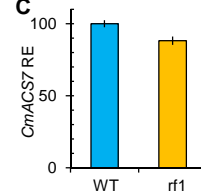
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	WT	<i>rf1</i>	<i>p</i> -value
ovary length (μm)	1962 \pm 87	1340 \pm 24	3.94 e-5
cell number in the longitudinal axis	144 \pm 8	135 \pm 5	ns
average cell length (μm)	13.6 \pm 0.6	10.1 \pm 0.5	1.26 e-5
ovary width (μm)	891 \pm 68	920 \pm 23	n.s
cell number in the transversal axis	94 \pm 9	117 \pm 9	0.003
average cell width (μm)	9.5 \pm 0.5	7.8 \pm 0.4	5.9 e-4
ovary shape index	2.21 \pm 0.2	1.5 \pm 0.03	5.8 e-4
cell shape index	1.44 \pm 0.1	1.3 \pm 0.09	0.026

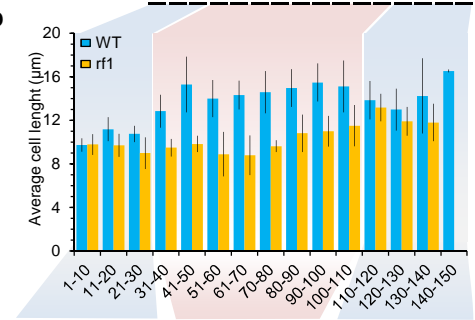
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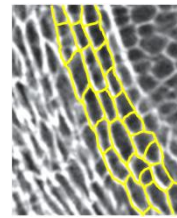
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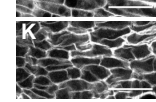
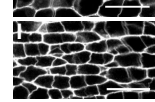
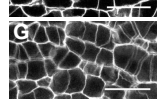
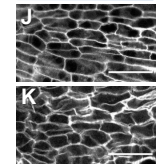
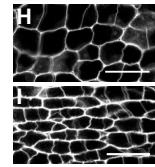
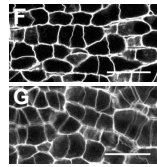
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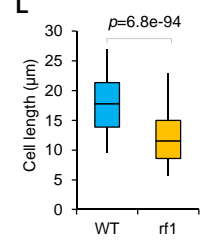
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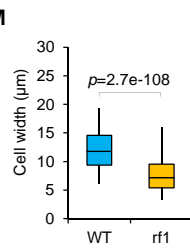
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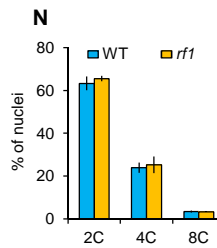
L



M



N



O

