

1 Unleashing meiotic crossovers in crops

2 Delphine Mieulet^{1,2}, Gregoire Aubert³, Cecile Bres⁴, Anthony Klein³, Gaëtan Droc^{1,2},
3 Emilie Vieille³, Celine Rond-Coissieux³, Myriam Sanchez³, Marion Dalmais⁵, Jean-
4 Philippe Mauxion⁴, Christophe Rothan⁴, Emmanuel Guiderdoni^{1,2} and Raphael
5 Mercier^{6*}

6 ¹ CIRAD, UMR AGAP, 34398 Montpellier Cedex 5, France

7 ² Univ Montpellier, CIRAD, INRA Montpellier SupAgro, Montpellier France

8 ³ Agroécologie, AgroSup Dijon, INRA, Univ. Bourgogne Franche-Comté, F-21000
9 Dijon, France

10 ⁴ UMR 1332 BFP, INRA, Univ. Bordeaux, F-33140 Villenave d'Ornon, France

11 ⁵ Institut of Plant Sciences, Paris Saclay IPS2, CNRS, INRA, Université Paris-Sud,
12 Université Evry, Université Paris-Saclay, 91405 Orsay, France

13 ⁶ Institut Jean-Pierre Bourgin, UMR1318 INRA-AgroParisTech, Université Paris-
14 Saclay, RD10, 78000 Versailles, France.

15 * Corresponding author. raphael.mercier@inra.fr

16

17

18 Improved plant varieties are hugely significant in our attempts to face the challenges
19 of a growing human population and limited planet resources. Plant breeding relies on
20 meiotic crossovers to combine favorable alleles into elite varieties¹. However, meiotic
21 crossovers are relatively rare, typically one to three per chromosome², limiting the
22 efficiency of the breeding process and related activities such as genetic mapping.
23 Several genes that limit meiotic recombination were identified in the model
24 species *Arabidopsis*². Mutation of these genes in *Arabidopsis* induces a large
25 increase in crossover frequency. However, it remained to be demonstrated whether
26 crossovers could also be increased in crop species hybrids. Here, we explored the
27 effects of mutating the orthologs of *FANCM*³, *RECQ4*⁴ or *FIGL1*⁵ on recombination in
28 three distant crop species, rice (*Oryza sativa*), pea (*Pisum sativum*) and tomato
29 (*Solanum lycopersium*). We found that the single *recq4* mutation increases
30 crossovers ~three-fold in these crops, suggesting that manipulating *RECQ4* may be
31 a universal tool for increasing recombination in plants. Enhanced recombination
32 could be used in combination with other state-of-the-art technologies such as
33 genomic selection, genome editing or speed breeding⁶ to enhance the pace and
34 efficiency of plant improvement.

35 Meiotic crossovers shuffle chromosomes to produce unique combinations of alleles
36 that are transmitted to offspring. Meiotic crossovers are thus at the heart of plant
37 breeding and any related genetic analysis such as quantitative trait loci (QTLs)
38 detection or gene mapping. However, crossovers are relatively rare events, which is
39 intriguing since their molecular precursors (*i.e.* DNA double stranded breaks and
40 inter-homologue joint molecules) largely outnumber the final crossover number.
41 Indeed, It was recently shown that active mechanisms limit the formation of meiotic
42 crossovers in *Arabidopsis*^{2-5,7-9}. Forward genetic screens identified three anti-

43 crossover pathways that rely on the activity of FANCM, RECQ4 and FIGL1,
44 respectively. RECQ4 appears to be the most important anti-crossover factor, as the
45 mutation of the corresponding genes (*RECQ4A* and *RECQ4B*) led to an almost four-
46 fold increase in recombination in *Arabidopsis* hybrids^{2,10}. RECQ4 is a DNA helicase
47 homologue of mammalian BLOOM and yeast Sgs1^{11,12}. *FANCM*, which encodes
48 another conserved DNA helicase, is also an important anti-crossover factor in
49 *Arabidopsis*. Mutation of this gene also leads to a large increase in recombination,
50 but only in pure lines (~3-fold) with a very limited effect in hybrids^{2,3,5,13}. *FANCM* was
51 also shown to limit crossovers in a *Brassica rapa* pure line¹⁴. The third pathway
52 depends on the AAA-ATPase FIGL1. Mutation in *FIGL1* alone leads to a relatively
53 modest increase in recombination (+25% in *Arabidopsis* hybrids), but when combined
54 with *recq4*² it leads to an almost eight-fold increase. Mutation in *FIGL1* leads to full
55 sterility in rice¹⁵, raising doubts about the pertinence of manipulating this gene in crop
56 species.

57 Here we tested the effect of *recq4*, *fancm* and *figl1* mutations on recombination in
58 three crop species. We chose rice (*Oryza sativa*), the cultivated pea (*Pisum sativum*)
59 and tomato (*Solanum lycopersium*) for their economic importance and because they
60 represent distant clades. Indeed they are members of the three major clades of
61 flowering plants, monocots, eudicots rosids and eudicots asterids, respectively¹⁶.
62 Rice is the staple of more than half of mankind and as such is the number one cereal
63 consumed. It belongs to the *Poaceae* family that also contains maize, wheat and
64 barley¹⁶. Pea, in addition to be the genetic model used by Mendel, is the second
65 most cultivated pulse crop in the world (<http://faostat.fao.org/>) and belongs to the
66 *Fabaceae* family that contains many crop species such as chickpea, beans and lentil.
67 Tomato, the second most cultivated fresh-market vegetable crop, is one of the most

68 important nutrient-dense superfoods and belongs to the *Solanaceae* family, which
69 includes potatoes, eggplant and peppers.

70 We first explored the conservation and copy number of *RECQ4*, *FANCM* and *FIGL1*
71 in flowering plants (Figures S1-3, Dataset S1). For *FANCM* and *FIGL1*, a single
72 homolog of each gene was identified in most species including pea, tomato and rice.
73 Several copies of *FANCM*¹⁷ and *FIGL1* were found only in very recent polyploids (e.g
74 wheat). Several copies of *RECQ4*, on the other hand, appear to have been retained
75 from earlier whole genome duplications in several clades, leading to the presence of
76 two or more copies in several species (e.g. *Arabidopsis*^{4,11}, cabbage, lettuce,
77 soybean, sunflower).

78 We then assessed the role of *OsRECQ4* (Os04g35420) and *OsFANCM*
79 (Os11g07870) in meiotic recombination in rice. We screened mutant collections of
80 two different cultivars, Nipponbare^{18,19} and Dongjin¹⁹, that are both from the
81 *japonica* temperate sub group. Comparison of 25X Illumina sequencing of Dongjin
82 and the Nipponbare reference genome, showed a divergence of one single
83 nucleotide polymorphism (SNP) per ~11kb (M&M). We identified one insertion mutant
84 in each cultivar for both genes (Figures 1A and S4). As mentioned above, mutation of
85 *FIGL1* was recently shown to cause sterility in rice and was thus not further studied
86 here¹⁵. We produced Dongjin/Nipponbare F1 hybrids mutant for both *OsRECQ4*
87 alleles, or for both *OsFANCM* alleles and wild type siblings (M&M and Figure S5).
88 Hybrid fertility was not affected by either the bi-allelic *Osfancm* or the *Osrecq4*
89 mutation (Figure 1B). No defects in meiosis progression were observed during male
90 meiosis in *Osrecq4* hybrids (Figure S6), which is consistent with normal fertility. F1
91 plants were self-fertilized to generate F2 populations that were genotyped for an

92 average of 19 SNP markers per chromosome (on the 12 chromosomes for the
93 *RECQ4* populations and five chromosomes for the *FANCM* populations). We
94 analyzed 149 *Osrecq4* *-/-* F2 plants, 108 *Osfancm* *-/-* F2 plants and a total of 262 wild
95 types (Dataset S2). In *Osrecq4* *-/-*, we observed an increase in the genetic size of all
96 12 chromosomes leading to a 3.2-fold increase in the total genetic map compared to
97 wild type (total size \pm 95% confidence interval: 5700 \pm 231 cM vs 1759 \pm 58 cM);
98 (Figures 2 and 3). This shows that RECQ4 is a major meiotic anti-crossover factor in
99 hybrid rice. In *Osfancm* *-/-*, recombination was increased by 2.3-fold (cumulated
100 genetic map size of the five chromosomes analyzed: 1649 \pm 122 cM vs 724 \pm 69 cM
101 in wild type). This is remarkable, as no increase in recombination was observed in
102 *Arabidopsis fancm* hybrids^{2,10} (Figures 2 and 3). Crossover distribution along the
103 chromosomes (Figure 4) showed that in both *Osfancm* and *Osrecq4*, enhancement
104 of recombination occurs along chromosome arms but not in the peri-centromeric
105 regions, suggesting that other factors limit crossovers in these regions, as previously
106 proposed for *Arabidopsis*. In addition to peri-centromeres, another region on the right
107 arm of chromosome 11 was relatively suppressed for crossovers in wild type and
108 *Osrecq4* (Figure 4). Interestingly, this region is associated with a cluster of resistance
109 genes²⁰ and diverges significantly between the parental genomes. The same
110 observation was made in *Arabidopsis*^{2,10}. This suggests that regions with high levels
111 of polymorphism are less prone to the extra crossovers that arise in *recq4* mutants.

112 Next, we extended our analysis to the pea *Pisum sativum* by screening an EMS-
113 induced mutant population²¹ (cultivar Cameor). We identified a STOP-codon mutation
114 in *PsFANCM*, *PsRECQ4* and a splicing site alteration in *PsFIGL1* (*fancm-Q503**,
115 *recq4-W673**, *figl1-L131ss*; Figure 1A). To measure the effect of these mutations
116 alone or in combination, we produced two independent populations. The first

117 population segregated the *Psrecq4* and *Psfancm* mutations and genetic
118 polymorphisms from a different cultivar (Kayanne) (M&M and Figure S7). The second
119 population was purely Cameor and segregated the three mutations (Figure S8).
120 Fertility was quite variable from plant to plant, presumably because of the
121 segregation of additional EMS mutations. In both populations, the fertility of F2
122 *Psfancm* mutants was indistinguishable from that of wild type. However, all the F2
123 plants that were homozygous for the *Psfigl1* mutation were sterile (Figure 1B) and
124 *Psrecq4* mutants produced four times less seed than wild type. This suggests that
125 PsFIGL1 is essential for meiosis and fertility in pea, as previously shown in rice¹⁵,
126 and that PsRECQ4 may also be required for full fertility (Figure 1B). However, we
127 cannot rule out the possibility that this reduced fertility in *Psrecq4* and *Psfigl1* was
128 caused by additional linked EMS mutations. Seeds could be obtained in sufficient
129 numbers for *Psrecq4*, *Psfancm*, *Psrecq4 Psfancm* and wild type siblings (Figure S7).
130 For each of these genotypes, ~50 F3 plants were genotyped for 5097 SNPs between
131 the cultivars Cameor and Kayanne (Dataset S4) to measure genome wide
132 recombination. Note that because certain regions were fixed in the F2s, only ~80% of
133 the genome was segregating for polymorphic markers in the four genotypes and was
134 thus analyzed to compare recombination levels (810 cM of the 1018 cM of the total
135 wild type map). For *Psfancm*, we observed a global twofold increase in recombination
136 (1639 ± 204 cM vs 810 ± 78 cM), similar to that observed in rice, but in contrast with
137 the absence of effect in Arabidopsis hybrids. In *Psrecq4*, recombination increased
138 even further with 4.7 times more crossovers observed compared to wild type ($3798 \pm$
139 296 cM vs 810 ± 78 cM) (Figures 2 and 3). Thus RECQ4 is a major anti-crossover
140 factor in Pea. *Psrecq4* and *Psfancm* double mutants did not show a further increase
141 in recombination compared to *Psrecq4* alone (3767 ± 288 cM vs 3798 ± 296 cM).

142 This suggests that in Pea either PsRECQ4 and PsFANCM act in the same anti-
143 crossover pathway, which would be intriguing as these two helicases appear to act in
144 parallel in Arabidopsis, or that some upper limit has been reached (e.g. the use of all
145 eligible crossover precursors).

146 Finally, we looked for mutations in *FANCM* and *RECQ4* in a tomato EMS-induced
147 mutant population (Cultivar Micro-Tom) (Figure 1A). We identified a STOP codon in
148 *SIRECQ4* (*recq4-Q511**) and crossed the corresponding line to wild type cultivar M82
149 (M&M and Figure S9). Wild type and *Sirecq4* F2 plants had similar fertility (Figure
150 1B). We focused our analysis on chromosome 4 and 7 and observed a 2.7-fold
151 increase in recombination in the mutant compared to the wild type (cumulative map
152 173 ± 22 cM vs 464 ± 52 cM) (Figures 2 and 3). This shows that RECQ4 is also a
153 major factor limiting meiotic recombination in tomato. We also identified missense
154 mutations in tomato *FANCM* in a conserved amino acid (L137F). Following a similar
155 approach as described above for *recq4*, we did not detect an increase in meiotic
156 recombination in hybrids homozygous for this mutation (data not shown). However,
157 further work is needed to understand whether disruption of *FANCM* has no effect in
158 this context, as observed for Arabidopsis hybrids, or if the L137F mutation does not
159 fully disrupt FANCM activity.

160 Discussion

161 Here we explored the potential for *fancm* and *recq4* mutation to increase
162 recombination in crops. In Arabidopsis, the *fancm* mutation leads to a threefold
163 increase in recombination in a pure line but has almost no effect in hybrids
164 (Col/Ler)^{5,13,22}. However, we showed here that mutating *FANCM* results in a ~twofold
165 increase in recombination in hybrid rice (Dongjin/Nipponbare) and hybrid pea

166 (Cameor/Kayanne). This difference could be due to variation in the recombination
167 machinery in these species or be associated with the level of polymorphisms in these
168 hybrids. Indeed, the SNP density is $\sim 1/200\text{pb}$ in the Col-Ler Arabidopsis hybrid²³, but
169 is much lower in the rice Dongjin/Nipponbare ($1/11\text{kb}$) and Cameor/Kayanne pea
170 hybrids ($\sim 1/10\text{kb}$ and $\sim 1/5\text{kb}$, respectively) and, by definition, virtually null in the
171 Arabidopsis pure line. This would mean that the *fancm* mutation only increases
172 recombination if the polymorphism rate is below a certain threshold, somewhere
173 between $1/200$ and $1/5000$ SNPs per kb. It would be interesting to explore the *fancm*
174 effect in more distant hybrids (e.g. *Japonica-Indica* rice) or in different species, to test
175 this hypothesis.

176 We showed that the *recq4* mutation alone can massively increase recombination in
177 rice, pea and tomato hybrids, a result similar to that observed in Arabidopsis². This
178 suggests that mutation in *RECQ4* orthologs may be a universal approach for
179 enhancing recombination rates in crop species. These increases in crossover
180 frequency are much higher than any previously observed natural or environmentally-
181 induced variation in recombination (e.g. temperature which typically modifies
182 recombination by 10-30%²⁴⁻²⁷). Increased recombination is predicted to improve the
183 response to selection in the short, medium, and long term²⁸. Thus higher
184 recombination rates could be used to enhance genetic gain in breeding schemes.
185 Further, increased recombination would also enhance the power of pre-breeding
186 activities such as genetic map construction, QTL detection, and positional cloning.
187 However, the *recq4* mutation does not homogeneously increase recombination along
188 the genome (Figure 3 and ²). First, the peri-centromeric regions, that are reluctant to
189 crossover in wild type, still fail to recombine in the mutants, suggesting that additional
190 unknown mechanisms prevent crossovers close to centromeres²⁹. Future studies

191 should prioritize the identification of these mechanisms and methods to increase
192 crossover in proximal regions as these regions represent a large part of the genome
193 in important crops such as wheat³⁰. Second, the increase in recombination tends to
194 be lower in more divergent regions of the genome. Strikingly, the regions of highest
195 sequence divergence showed a limited increase in recombination compared to the
196 rest of the genome (Figure 4 and ²). This suggests that the extra crossovers arising in
197 the *recq4* or *fancm* mutants tend to be prevented by sequence divergence. This
198 predicts that mutating *recq4* could be ineffective for promoting recombination
199 between distant genomes, such as in interspecific crosses, but this remains to be
200 tested. The same appears to be true for all anti-crossover genes identified to date^{7,10}.
201 Further studies are required to understand how sequence divergence drives genetic
202 recombination.

203 In all species examined so far, mutation in *RECQ4* resulted in the most significant
204 increases in crossover numbers. However in Arabidopsis, further increases were
205 obtained by combining the *recq4* mutation with either a mutation in the *FIGL1* gene,
206 or with overexpression of HEI10^{2,10}. While *figl1* only mildly affects fertility in
207 Arabidopsis, it leads to sterility in rice¹⁵ and pea, precluding the use of *figl1* to
208 manipulate recombination in those species. Both *figl1* mutation and HEI10
209 overexpression remain to be tested in other species.

210 Here we used classic mutagenesis to disrupt *FANCM* and *RECQ4* and crosses to
211 introduce this mutation into the hybrid context. However, the development of very
212 effective targeted mutagenesis techniques based on CRISPR-cas9 now offers the
213 possibility to disrupt these genes directly in the F1 hybrids³¹ and thus rapidly obtain
214 hyper-recombined populations and enhance the efficiency of crop breeding.

215 **Materials and methods**

216 **Phylogeny**

217 Sequences from RECQ4, FANCM and FIGL1 proteins were retrieved from the
218 PLAZA V4 dicots and monocots databases³²
219 <https://bioinformatics.psb.ugent.be/plaza/> using BLASTP (RECQ4:
220 ORTH004M000654 and ORTH004D00423; FANCM ORTH004D004865 and
221 ORTH004M004526) and species by species using BLASTP on the nr database at
222 NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins>), Phytosome12
223 (<https://phytozome.jgi.doe.gov/pz/portal.html>), the Pea RNA-Seq gene atlas
224 (<http://bios.dijon.inra.fr/FATAL/cgi/pscaml.cgi>)²¹, the Sol Genomics Network
225 <https://solgenomics.net/>³³ and the IWGSC RefSeq Annotations. For each candidate
226 gene, if several protein isoforms/predictions were present in the databases, the
227 isoform/prediction with the higher similarity to the corresponding protein in other
228 species was retained for further analysis (Dataset S1). The phylogenetic analysis
229 was performed on the Phylogeny.fr platform³⁴ and included the following steps: 1)
230 Sequences were aligned with MUSCLE (v3.8.31) configured for highest accuracy; 2)
231 Positions with gaps were removed from the alignment; 3) The phylogenetic tree was
232 reconstructed using the maximum likelihood method implemented in the PhyML
233 program (v3.1/3.0 aLRT). The default substitution model was selected assuming an
234 estimated proportion of invariant sites (of 0.118) and four gamma-distributed rate
235 categories to account for rate heterogeneity across sites. The gamma shape
236 parameter was estimated directly from the data (gamma=0.929). Reliability of internal
237 branches was assessed using the aLRT test (SH-Like). Graphical representation and

238 editing of the phylogenetic tree were performed with TreeDyn (v198.3) and adobe
239 illustrator.

240 **Rice**

241 Illumina Paired-end reads from Dongjin were aligned to the Nipponbare reference
242 genome (MSU7) using the software BWA (release 0.7.10). PCR artifacts were
243 removed by Picard tools MarkDuplicates (<https://broadinstitute.github.io/picard/>). SNP
244 and INDEL identification were performed with GATK HaplotypeCaller (release 3.4-0-
245 g7e26428) with default parameter. Raw variants were filtered according to GATK
246 recommendations (<https://software.broadinstitute.org/gatk/best-practices/>). In
247 resulting VCF file (Variant Call Format) we retained only variants that have passed all
248 filters (PASS quality) and we selected homozygous SNPs (both alleles are different
249 from those of Nipponbare reference). 33540 SNPs were retained for a total genome
250 size of 373 Mb, corresponding to 1 SNP per 11 Kb between Dongjin and Nipponbare
251 cultivars.

252 The following mutations were used in this study: *Osfancm-1* (AQSG07), *Osfancm-2*
253 (A46543), *Osrecq4l-1* (3A-03503) and *Osrecq4l-3* (AUF12) (Figure 1A and Figure
254 S4). *Osfancm-1* and *Osrecq4l-3* are in the Nipponbare cultivar from the Oryza Tag
255 Line insertion line library^{18,35}. *Osfancm-2* and *Osrecq4l-1* are in the Dongjin cultivar
256 from the POSTECH Rice Insertion Database³⁶. For each allele, the position of the T-
257 DNA in the rice genome was confirmed with Sanger sequencing (Figure S4). Plants
258 were grown under containment greenhouse conditions (28°C / 24°C day/night cycle,
259 60% humidity) with natural light boosted by artificial sodium lights (light intensity of
260 700 $\mu\text{moles/m}^2/\text{s}$). The crossing scheme is summarized in figure S5. Heterozygous
261 plants for the mutations were identified using PCR. Primers were designed using the

262 “Genotyping Primer Designer” tool of OryGenesDB
263 (<http://www.orygenesdb.cirad.fr>)^{35,37}. Genotyping primers and expected PCR product
264 sizes are listed in Table S1. We crossed the heterozygous lines *Osfancm-2+/-* with
265 *Osfancm-1+/-* and *Osrecq4-1+/-* with *Osrecq4-3+/-*. Dongjin lines were used as
266 female and Nipponbare lines as male (Figure S5). Crosses were carried out through
267 manual castration of florets and pollination, followed by bagging to avoid pollen
268 contamination. F1 hybrid plants were genotyped twice to select *Osfancm-/-*, *Osrecq4-*
269 *-/-* and their respective wild type siblings (Figure S5). F1 sibling plants of the desired
270 genotypes were used for fertility measurements, cytological analyses and selfed to
271 produce the F2 populations. Male meiotic chromosome spreads were performed as
272 previously described³⁸. For SNPs genotyping of the F2s, DNA was extracted from
273 500mg of fresh leaves and adjusted to 10ng/ μ L. Single nucleotide polymorphism
274 genotyping was performed using Kompetitive Allele Specific PCR (KASP) following
275 the LGC group recommendations for the use of KASP technology on Biomark
276 Fluidigm with a set of 241 robust KASP markers spread over the physical map
277 (~every 1.5 Mb). Genotyping data were analyzed with Fluidigm software (Fluidigm
278 SNP Genotyping Analysis 4.3.2) with manual error corrections. The raw genotyping
279 dataset is shown in Dataset S2. Recombination analysis was performed with
280 MapDisto 2.0 b105³⁹. Linkage groups were determined for the wild type F2
281 population (LOD1, RFmax: 0,5), and fit perfectly with the physical marker order.
282 Genotyping errors were filtered using the iterative error removal function (iterations =
283 5, start threshold = 0.001, increase = 0.001). Recombination (cM \pm SEM) was
284 calculated using classical fraction estimation and the Haldane mapping function. The
285 obtained recombination frequencies per interval and corresponding genomic data are
286 shown in Dataset S3. Graphical representations were generated with R 3.3.2 (Figure

287 4).

288 **Pea**

289 Mutations in *PsRECQ4*, *PsFANCM* and *PsFIGL1* were identified using TILLING
290 (Targeting Induced Local Lesions IN Genome) in the cultivar Cameor, and combined
291 by crosses. In the *Psfancm* mutant there is a C to T transition at position 1507 from
292 the A of the start codon of the coding sequence, leading to a nonsense mutation
293 (Q503*). In *Psrecq4* there is a G to A transition at the position 2019 from the A of the
294 start codon of the coding sequence, leading to a nonsense mutation (W673*).

295 The *PsFigl1* mutation is a G to A transition at position 3740 from the ATG on the
296 genomic sequence, modifying the splice junction before the 3rd exon. Two
297 independent populations were produced (Figures S7 and S8).

298 In the first population, one plant *PsRECQ4+/- PsFANCM+/-* was crossed to the wild
299 type cultivar Kayanne (Figure S7). One F1 plant was selfed to produce 180 F2 plants,
300 among which single mutants, double mutants and wild type were identified by
301 genotyping. Five *Psfancm*, five *Psrecq4*, three *Psfancm Psrecq4* and five wild type
302 F2 plants were selfed to produce the F3 populations (~50 plants per genotype).

303 In the second population, two Cameor *PsRECQ4+/- PsFANCM+/- PsFIGL1+/-* were
304 selfed to produce 160 F2 plants (Figure S8). Twenty-one *Psfancm* mutants, 24
305 *Psrecq4*, 24 *Psfigl1*, 2 *Psfigl1Psrecq4* double mutants and 7 *Psfigl1Psfancm* double
306 mutants were identified by genotyping. Fertility was analyzed for the two F2
307 populations (Figure 1 B).

308 F2 and F3 plants of the Cameor/Kayanne hybrid population were genotyped for 5097
309 markers polymorphic between Kayanne and Cameor using the GenoPea 13.2K SNP
310 Array⁴⁰ (Dataset S4). Markers that were homozygous in F2 plants were scored as

311 missing data in its F3 progeny. Very rare dubious singletons were manually edited
312 into missing data. Recombination analysis was carried out with MapDisto 2.0 b104³⁹,
313 using the linkage groups defined in⁴⁰ with some manual corrections that minimized
314 the number of crossovers. The F2 and F3 wild type maps were not significantly
315 different from each other and were combined to gain detection power. Recombination
316 (cM \pm SEM) was calculated using classical fraction estimation and the Haldane
317 mapping function. The obtained recombination frequencies per interval and
318 corresponding genomic data are shown in Dataset S5. Complete maps are shown in
319 figure 2. Only the genetic space for which data were obtained in the four genotypes
320 (~80% of the total map) is shown in figure 3 (common map in Dataset S5).

321

322 **Tomato**

323 Q511>STOP *RECQ4* and L137F *FANCM* mutations were isolated using TILLING in a
324 tomato EMS mutant collection in the cultivar Micro-Tom^{41,42}. Genetic mapping was
325 carried out in F3 populations from a cross between a *recq4-Q511** homozygous
326 mutant and the processing variety M82 (Figure S9). A 96 F2 population from a F1
327 hybrid was genotyped for the *recq4* mutation using a set of 30 markers on
328 chromosomes 4 and 7 that are polymorphic between Micro-Tom and M82^{43,44}
329 (Dataset S6). A total of 16 F2 plants were selected for their maximal heterozygosity
330 for chromosome 4 or chromosome 7 and for being either *RECQ4+/+* or *recq4/-*
331 (Figure S9). Forty F3 progenies were generated by selfing from each of these F2
332 plants. The 640 F2 plants were genotyped for SNP markers on chromosome 4 or 7.
333 The plants were grown and DNA extracted as described in^{43,45}. Genotyping was
334 performed by KASPTM Assay⁴⁶. Markers that were homozygous in F2 plants were

335 scored as missing data in its F3 progeny. Recombination analyses were performed
336 with MapDisto 2.0 b104⁴⁷. Genotyping errors were filtered using the iterative error
337 removal function (iterations = 1, start threshold = 0.001). Recombination (cM \pm SEM)
338 was calculated using classical fraction estimation and the Haldane mapping function.
339 The obtained recombination frequencies per interval are shown in Dataset S7.

340

341 **Acknowledgment**

342 We thank Judith Burstin, Mathilde Causse, Brigitte Courtois and Christine Mézard for
343 fruitful discussions. We thank Pierre Sourdille and Fatiha Benyahya for sharing wheat
344 sequences before publication. We thank Christine Le Signor and Marie-Christine Le
345 Paslier for offering their expertise and advices. The pea and tomato work was funded
346 by the HyperRec grants from INRA Transfert. The Institute Jean-Pierre Bourgin
347 benefits from the support of the LabEx Saclay Plant Sciences-SPS (ANR-10-LABX-
348 0040-SPS). This work was partly funded by the "Investissements d'Avenir", France
349 Génomique project IRIGIN (INTERNATIONAL RICE GENOME INITIATIVE).

350 **Conflict of interest.**

351 Patents have been deposited by INRA on the use of RECQ4, FIGL1, and FANCM to
352 manipulate meiotic recombination (EP3149027, EP3016506, and EP2755995).

353

354 **References**

- 355 1. Wijnker, E. & de Jong, H. Managing meiotic recombination in plant breeding.
356 *Trends Plant Sci.* **13**, 640–6 (2008).
- 357 2. Fernandes, J. B., Seguéla-Arnaud, M., Larchevêque, C., Lloyd, A. H. &
358 Mercier, R. Unleashing meiotic crossovers in hybrid plants. *Proc. Natl. Acad.*
359 *Sci. U. S. A.* 201713078 (2017). doi:10.1073/pnas.1713078114
- 360 3. Crismani, W. *et al.* FANCM Limits Meiotic Crossovers. *Science.* **336**, 1588–
361 1590 (2012).
- 362 4. Séguéla-Arnaud, M. *et al.* Multiple mechanisms limit meiotic crossovers:

- 363 TOP3 α and two BLM homologs antagonize crossovers in parallel to FANCM.
364 *Proc. Natl. Acad. Sci. U. S. A.* **112**, 4713–4718 (2015).
- 365 5. Girard, C. *et al.* AAA-ATPase FIDGETIN-LIKE 1 and helicase FANCM
366 antagonize meiotic crossovers by distinct mechanisms. *PLoS Genet.* **11**,
367 e1005369 (2015).
- 368 6. Watson, A. *et al.* Speed breeding is a powerful tool to accelerate crop research
369 and breeding. *Nat. plants* **4**, 23–29 (2018).
- 370 7. Ziolkowski, P. A. *et al.* Natural variation and dosage of the HEI10 meiotic E3
371 ligase control Arabidopsis crossover recombination. *Genes Dev.* 1–12 (2017).
372 doi:10.1101/gad.295501.116
- 373 8. Séguéla-Arnaud, M. *et al.* RMI1 and TOP3 α limit meiotic CO formation through
374 their C-terminal domains. *Nucleic Acids Res.* **45**, 1860–1871 (2017).
- 375 9. Girard, C. *et al.* FANCM-associated proteins MHF1 and MHF2, but not the
376 other Fanconi anemia factors, limit meiotic crossovers. *Nucleic Acids Res.* **42**,
377 9087–9095 (2014).
- 378 10. Serra, H. *et al.* Massive crossover elevation via combination of HEI10 and recq4a
379 recq4b during Arabidopsis meiosis. *Proc. Natl. Acad. Sci. U. S. A.* **115**, 2437–
380 2442 (2018).
- 381 11. Hartung, F. & Puchta, H. The RecQ gene family in plants. *J. Plant Physiol.* **163**,
382 287–296 (2006).
- 383 12. Hartung, F., Suer, S. & Puchta, H. Two closely related RecQ helicases have
384 antagonistic roles in homologous recombination and DNA repair in Arabidopsis
385 thaliana. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 18836–41 (2007).
- 386 13. Ziolkowski, P. A. *et al.* Juxtaposition of heterozygous and homozygous regions

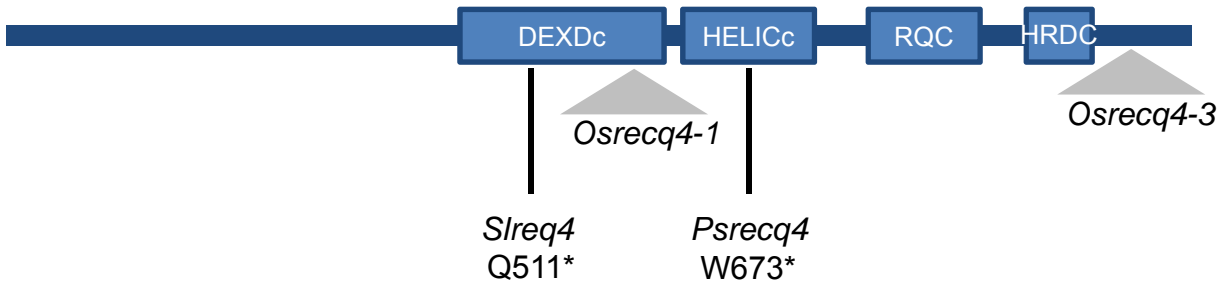
- 387 causes reciprocal crossover remodelling via interference during Arabidopsis
388 meiosis. *Elife* **4**, 1–29 (2015).
- 389 14. Blary, A. *et al.* FANCM Limits Meiotic Crossovers in Brassica Crops. *Front.*
390 *Plant Sci.* **9**, 1–13 (2018).
- 391 15. Zhang, P. *et al.* The rice AAA-ATPase OsFIGNL1 is essential for male meiosis.
392 *Front. Plant Sci.* **8**, 1639 (2017).
- 393 16. Chase, M. W. *et al.* An update of the Angiosperm Phylogeny Group
394 classification for the orders and families of flowering plants: APG IV. *Bot. J.*
395 *Linn. Soc.* **181**, 1–20 (2016).
- 396 17. Lloyd, A. H. *et al.* Meiotic gene evolution: can you teach a new dog new tricks?
397 *Mol. Biol. Evol.* **31**, 1724–7 (2014).
- 398 18. Sallaud, C. *et al.* High throughput T-DNA insertion mutagenesis in rice: a first
399 step towards in silico reverse genetics. *Plant J.* **39**, 450–64 (2004).
- 400 19. Larmande, P. *et al.* Oryza Tag Line, a phenotypic mutant database for the
401 Génoplante rice insertion line library. *Nucleic Acids Res.* **36**, 1022–1027
402 (2008).
- 403 20. Zhou, T. *et al.* Genome-wide identification of NBS genes in japonica rice
404 reveals significant expansion of divergent non-TIR NBS-LRR genes. *Mol.*
405 *Genet. Genomics* **271**, 402–415 (2004).
- 406 21. Alves-Carvalho, S. *et al.* Full-length de novo assembly of RNA-seq data in pea
407 (*Pisum sativum* L.) provides a gene expression atlas and gives insights into
408 root nodulation in this species. *Plant J.* **84**, 1–19 (2015).
- 409 22. Fernandes, J. B. *et al.* FIGL1 and its novel partner FLIP form a conserved
410 complex that regulates homologous recombination. *PLOS Genet.* **14**,

- 411 e1007317 (2018).
- 412 23. Zapata, L. *et al.* Chromosome-level assembly of *Arabidopsis thaliana* Ler
413 reveals the extent of translocation and inversion polymorphisms. *Proc. Natl.*
414 *Acad. Sci. U. S. A.* **113**, E4052-60 (2016).
- 415 24. Phillips, D. *et al.* The effect of temperature on the male and female
416 recombination landscape of barley. *New Phytol.* n/a-n/a (2015).
417 doi:10.1111/nph.13548
- 418 25. Francis *et al.* Pollen tetrad-based visual assay for meiotic recombination in
419 *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 3913–8 (2007).
- 420 26. Lloyd, A. H., Morgan, C., Franklin, C. & Bomblies, K. Plasticity of Meiotic
421 Recombination Rates in Response to Temperature in *Arabidopsis*. *Genetics*
422 genetics.300588.2017 (2018). doi:10.1534/genetics.117.300588
- 423 27. Modliszewski, J. L. *et al.* Elevated temperature increases meiotic crossover
424 frequency via the interfering (Type I) pathway in *Arabidopsis thaliana*. *PLoS*
425 *Genet.* **14**, e1007384 (2018).
- 426 28. Wilfert, L., Gadau, J. & Schmid-Hempel, P. Variation in genomic recombination
427 rates among animal taxa and the case of social insects. *Heredity (Edinb)*. **98**,
428 189–197 (2007).
- 429 29. Nambiar, M. & Smith, G. R. Repression of harmful meiotic recombination in
430 centromeric regions. *Semin. Cell Dev. Biol.* **54**, 188–197 (2016).
- 431 30. Choulet, F. *et al.* Structural and functional partitioning of bread wheat
432 chromosome 3B. *Science* **345**, 1249721 (2014).
- 433 31. Yin, K., Gao, C. & Qiu, J.-L. Progress and prospects in plant genome editing.
434 *Nat. Plants* **3**, 17107 (2017).

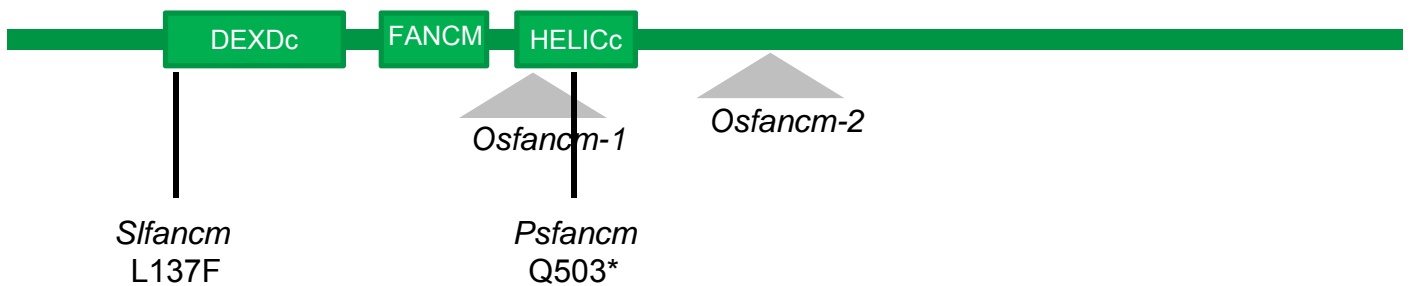
- 435 32. Van Bel, M. *et al.* PLAZA 4.0: an integrative resource for functional,
436 evolutionary and comparative plant genomics. *Nucleic Acids Res.* **46**, D1190–
437 D1196 (2018).
- 438 33. Fernandez-Pozo, N. *et al.* The Sol Genomics Network (SGN)—from genotype
439 to phenotype to breeding. *Nucleic Acids Res.* **43**, D1036–D1041 (2015).
- 440 34. Dereeper, A. *et al.* Phylogeny.fr: robust phylogenetic analysis for the non-
441 specialist. *Nucleic Acids Res.* **36**, W465-9 (2008).
- 442 35. Droc, G. *et al.* OryGenesDB: a database for rice reverse genetics. *Nucleic*
443 *Acids Res.* **34**, D736-40 (2006).
- 444 36. An, S. *et al.* Generation and analysis of end sequence database for T-DNA
445 tagging lines in rice. *Plant Physiol.* **133**, 2040–2047 (2003).
- 446 37. Droc, G., Périn, C., Fromentin, S. & Larmande, P. OryGenesDB 2008 update:
447 database interoperability for functional genomics of rice. *Nucleic Acids Res.* **37**,
448 D992-5 (2009).
- 449 38. Grelon, M., Vezon, D., Gendrot, G. & Pelletier, G. AtSPO11-1 is necessary for
450 efficient meiotic recombination in plants. *EMBO J.* **20**, 589–600 (2001).
- 451 39. Heffelfinger, C., Fragoso, C. A. & Lorieux, M. Constructing linkage maps in the
452 genomics era with MapDisto 2.0. *Bioinformatics* **33**, 2224–2225 (2017).
- 453 40. Tayeh, N. *et al.* Development of two major resources for pea genomics: The
454 GenoPea 13.2K SNP Array and a high-density, high-resolution consensus
455 genetic map. *Plant J.* **84**, 1257–1273 (2015).
- 456 41. Baldet, P. *et al.* Investigating the role of vitamin C in tomato through TILLING
457 identification of ascorbate-deficient tomato mutants. *Plant Biotechnology* **30**,
458 309–314 (2013).

- 459 42. Okabe, Y. *et al.* Tomato TILLING technology: Development of a reverse
460 genetics tool for the efficient isolation of mutants from micro-tom mutant
461 libraries. *Plant Cell Physiol.* **52**, 1994–2005 (2011).
- 462 43. Garcia, V. *et al.* Rapid identification of causal mutations in tomato EMS
463 populations via mapping-by-sequencing. *Nat. Protoc.* **11**, 2401–2418 (2016).
- 464 44. Shirasawa, K., Hirakawa, H., Nunome, T., Tabata, S. & Isobe, S. Genome-wide
465 survey of artificial mutations induced by ethyl methanesulfonate and gamma
466 rays in tomato. *Plant Biotechnol. J.* **14**, 51–60 (2016).
- 467 45. Petit, J. *et al.* Analyses of Tomato Fruit Brightness Mutants Uncover Both
468 Cutin-Deficient and Cutin-Abundant Mutants and a New Hypomorphic Allele of
469 GDSL Lipase. *PLANT Physiol.* **164**, 888–906 (2014).
- 470 46. Smith, S. M. & Maughan, P. J. SNP genotyping using KASPar assays.
471 *Methods Mol. Biol.* **1245**, 243–256 (2015).
- 472 47. Lorieux, M. MapDisto: fast and efficient computation of genetic linkage maps.
473 *Mol. Breed.* **30**, 1231–1235 (2012).
- 474
- 475

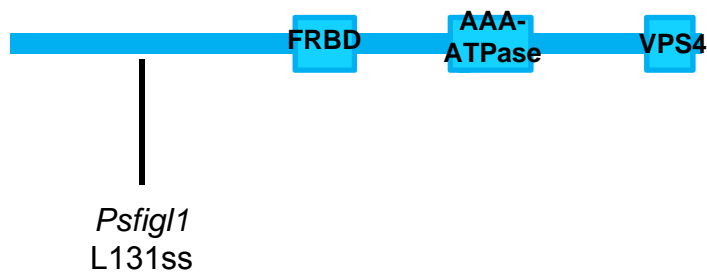
RECQ4



FANCM



FIGL1



100 aa

Figure 1A. Graphical representation of the RECQ4, FANCM and FIGL1 proteins and positions of the mutations described in this study for rice (*Os* for *Oryza sativa*), tomato (*Sl* for *Solanum lycopersicum*) and pea (*P*s for *Pisum sativum*). T-DNA insertions are indicated with a triangle and EMS point mutations with a black vertical line. Conserved Protein domains are represented by rectangles. AAA-ATPase : ATPase Associated with diverse cellular Activities; DEXDc : DEAD-like helicase domain; FANCM : Fanconi anemia complementation group M; FRBD : FIDGETIN-RAD51-Binding-Domain; HELICc : Helicase superfamily C-terminal domain; HRDC : Homologous region RNase D C-terminal; RQC : RecQ C-terminal; VPS4 : Vacuolar Protein Sorting 4.

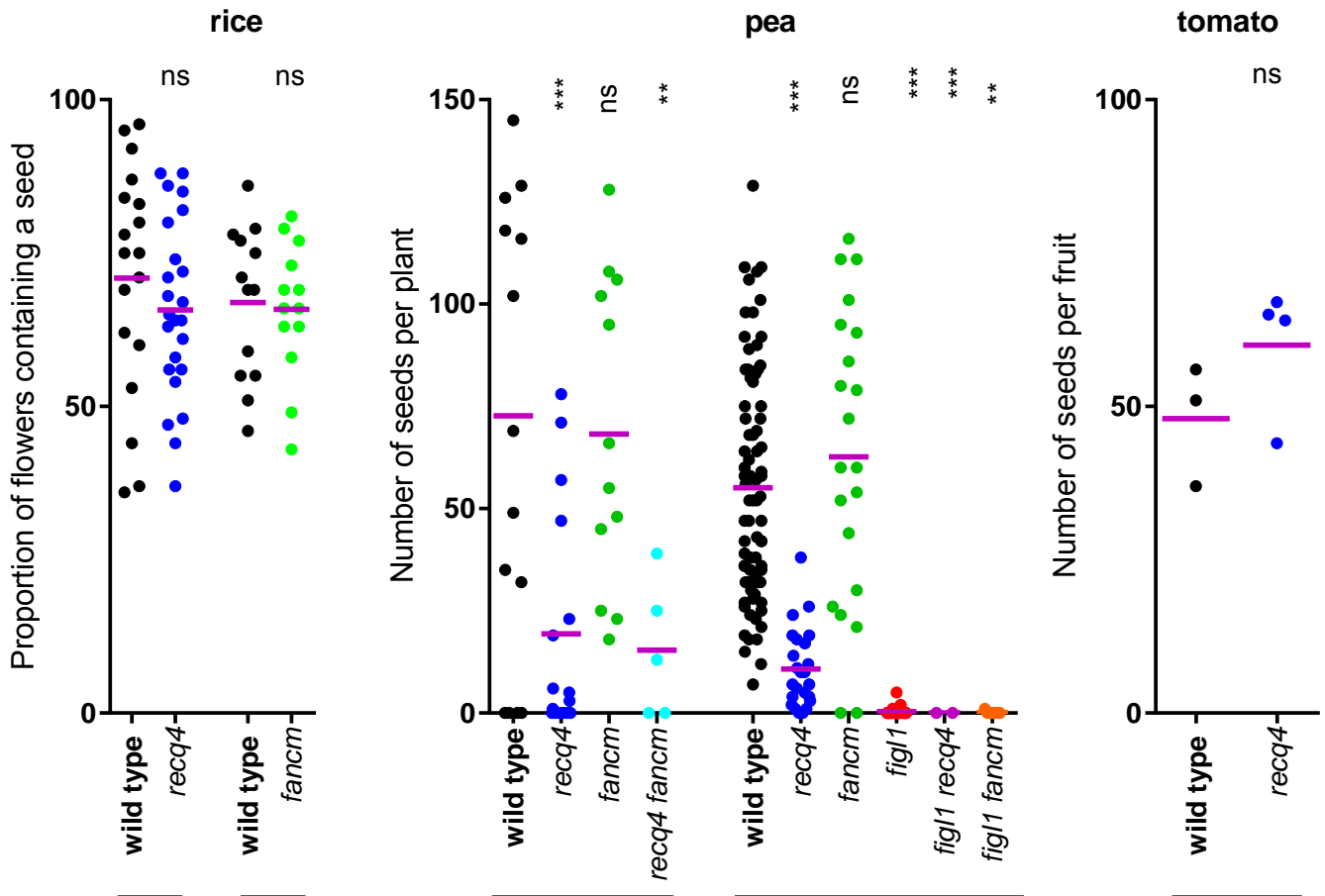


Figure 1B. Fertility Analysis of the *recq4*, *fancm* and *fig1* mutants in rice, pea and tomato. For rice, each dot represents the fertility of an individual plant measured as the proportion of flowers giving rise to a seed ($n > 150$ flowers/plant). For pea each dot represents the fertility of an individual plant measured as the total number of seeds per plant. For tomato each dot represents the fertility of an individual plant measured as the number of seeds per fruit ($n = 3$ fruits per plant). The bar under the graph indicates that the plants are siblings. The purple bars represent the mean. Anova with Sidak's multiple comparison correction: *** $p < 0.001$; ** $p < 0.01$; not significant (ns) $p > 0.05$.

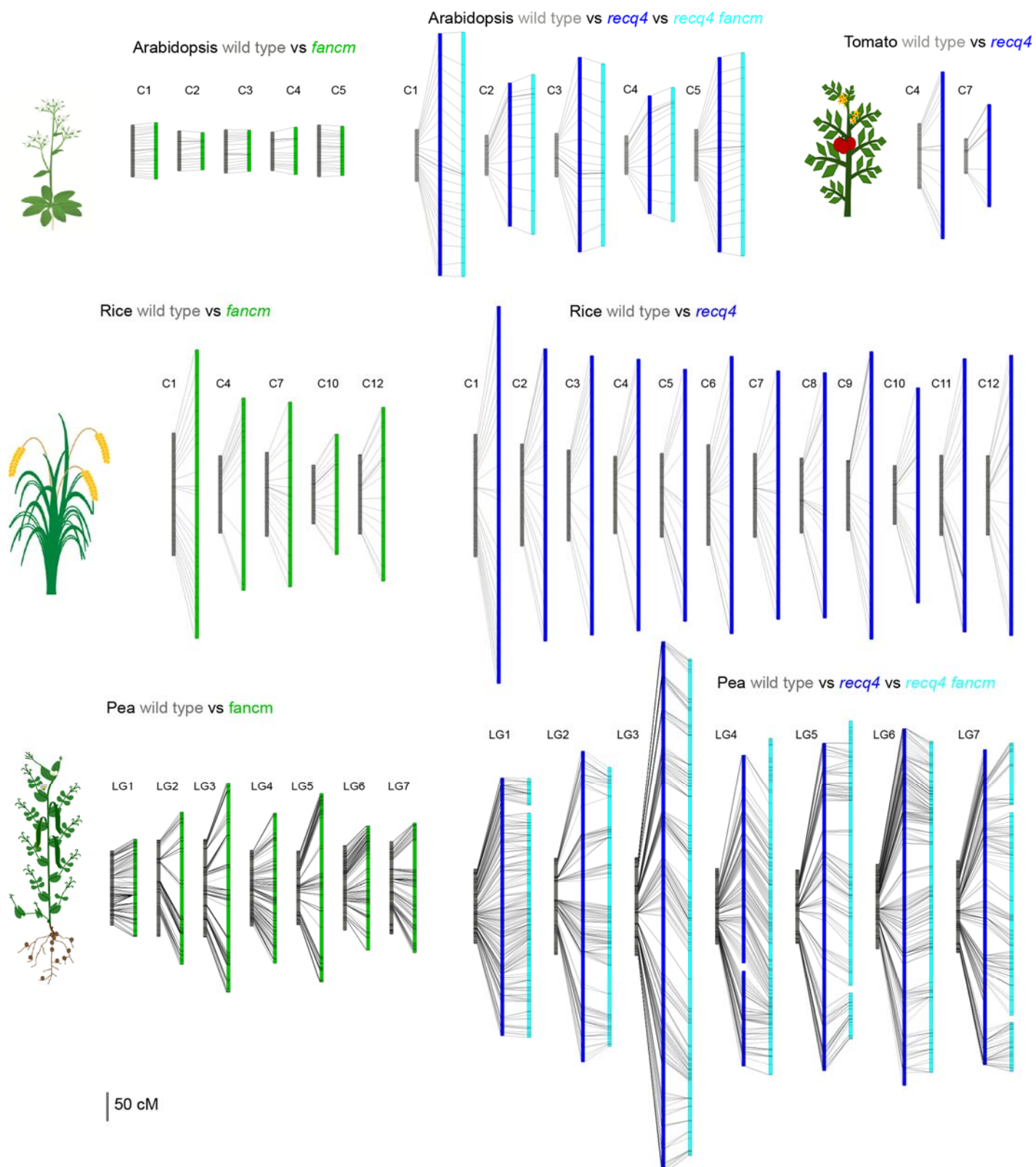


Figure 2. Genetic maps in *fancm* and *recq4* mutants compared with wild type for Arabidopsis, rice, pea and tomato.

C=chromosome. LG=Linkage group. Each black line represents an informative genetic marker. Data can be found in Tables S3, S5 and S7. Data for Arabidopsis are from Fernandes et al ²

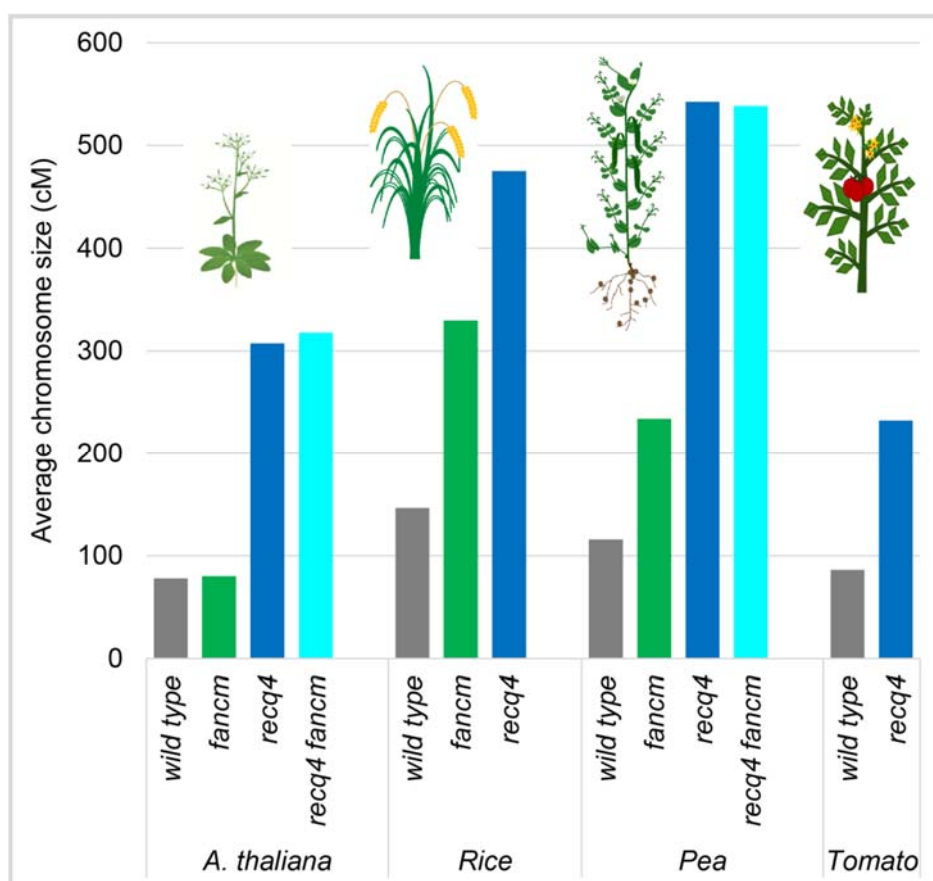


Figure 3. Average chromosome size in wild type, *fancm* and *recq4* mutant plants for Arabidopsis, rice, pea and tomato.

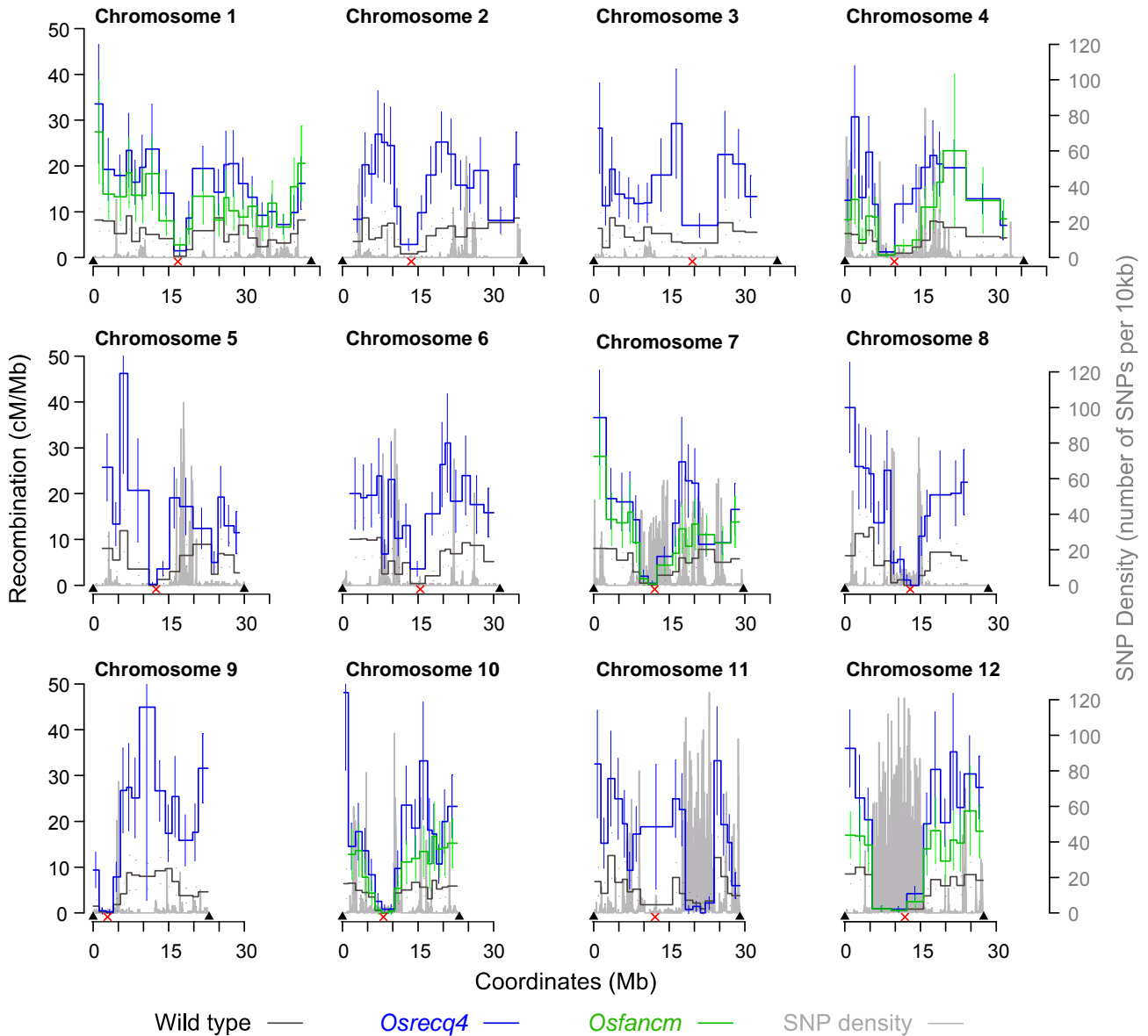


Figure 4. Distribution of COs along the 12 rice chromosomes in *Osrecq4I* (blue), *Osfancm* (green) and wild type (grey) plants. The recombination frequency (cM/Mb) in each interval was plotted along the 12 rice chromosomes. The density of SNP polymorphisms between Dongjin and Nipponbare strains is shown in grey. Red crosses represent the centromere positions ; the arrows represent the telomere positions.

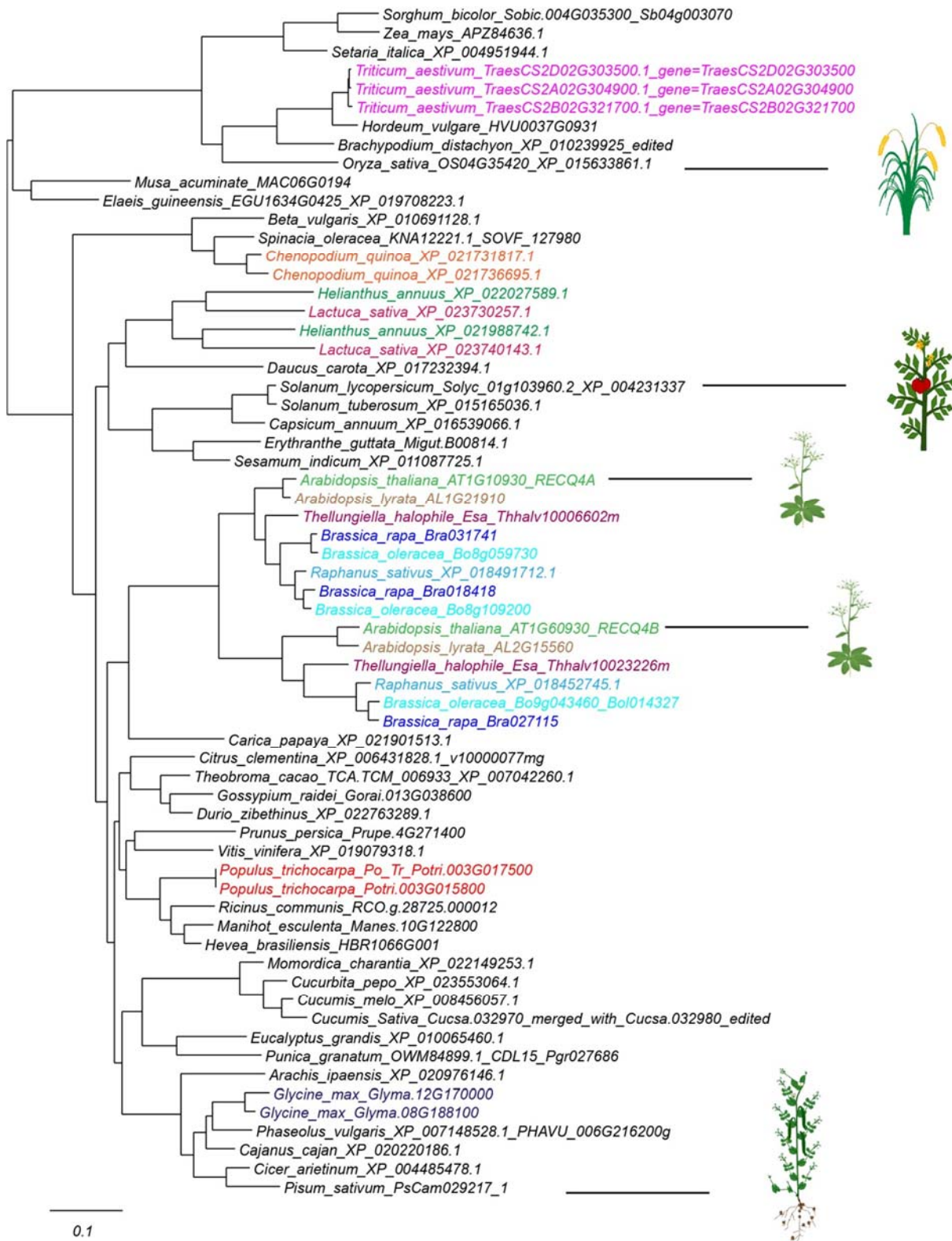


Figure S1. Phylogenetic tree of plant RECQ4 proteins. Genes present in several copies in a given species have been colored. Proteins sequences and accession numbers can be found in dataset S1



Figure S2. Phylogenetic tree of plant FANCM proteins. Genes present in several copies in a given species have been colored. Proteins sequences and accession numbers can be found in dataset S1

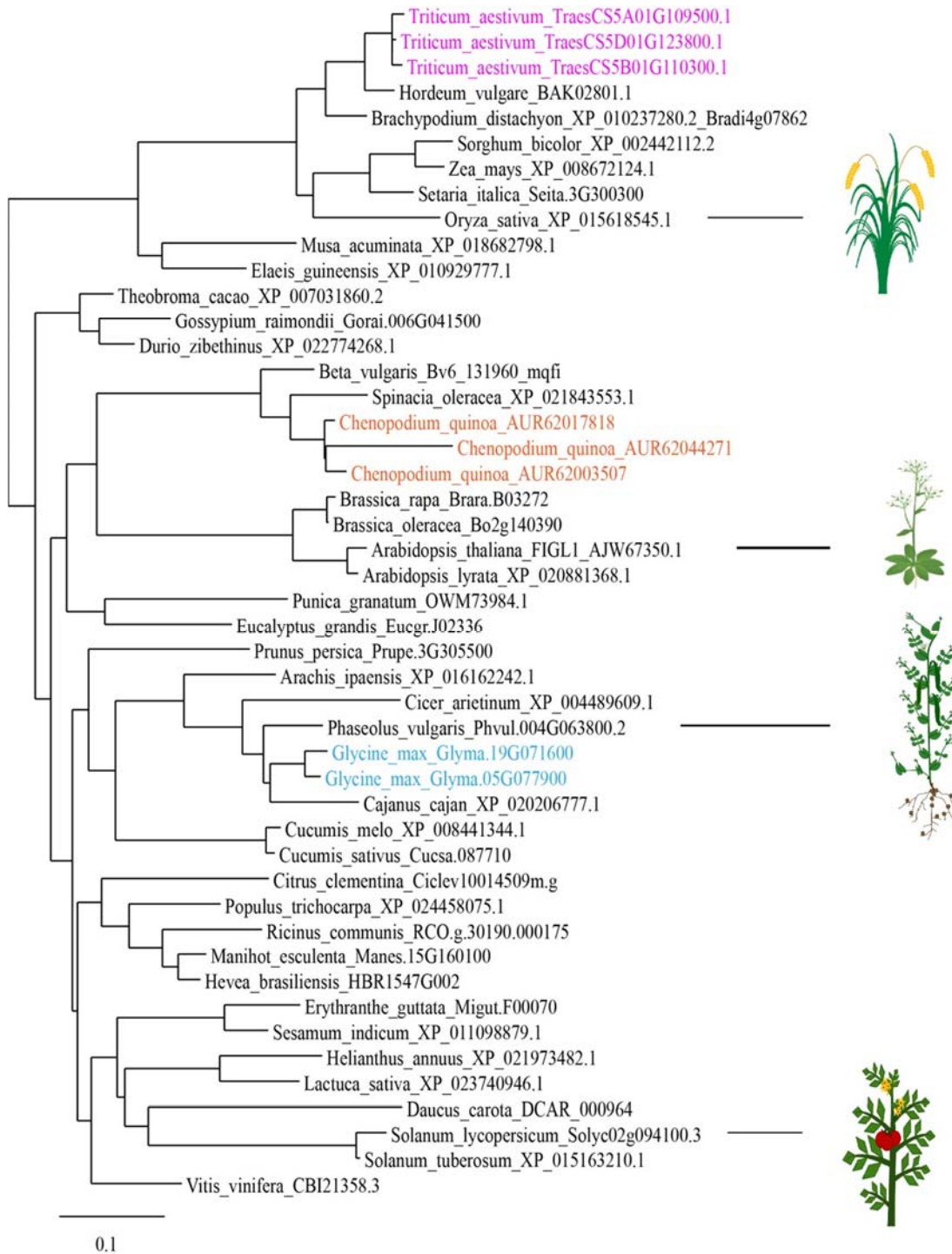
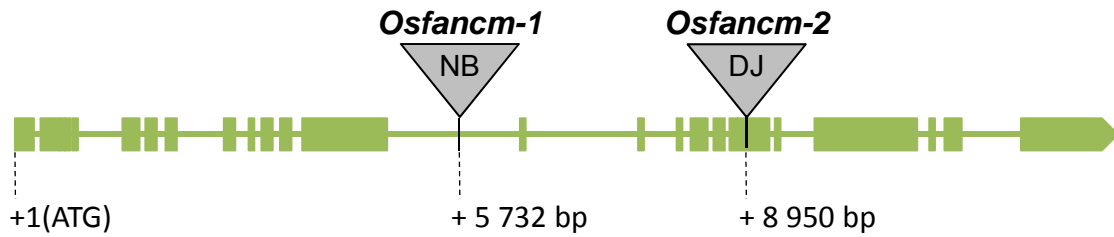


Figure S3. Phylogenetic tree of FIGL1. Genes present in several copies in a given species have been colored. Proteins sequences and accession numbers can be found in dataset S1

Os11g07870; *OsFANCM* (ADNg = 12 347 bp; cDNA = 4 467 pb)

1Kb



Os04g35420; *OsRECQ4* (ADNg = 11 282bp; cDNA = 3 523 pb)

1Kb

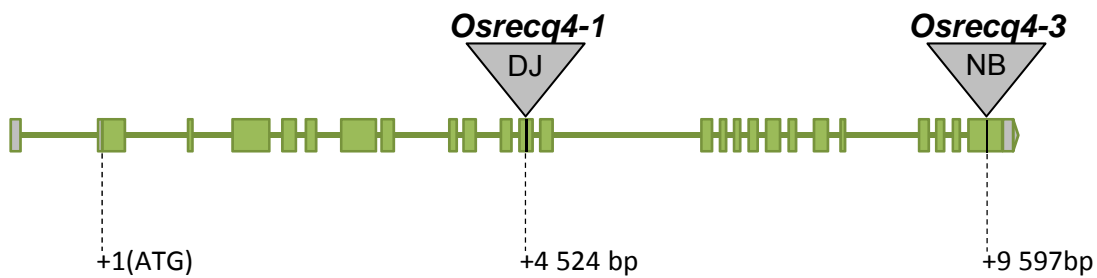


Figure S4 : Positions of T-DNA insertions in *OsFANCM* and *OsRECQ4*. T-DNA insertions are indicated with a triangle. Mutants are from two different cultivars, Nipponbare (NB) or Dongjin (DJ). The exact position of the T-DNA insertion site was confirmed by Sanger sequencing.

Rice

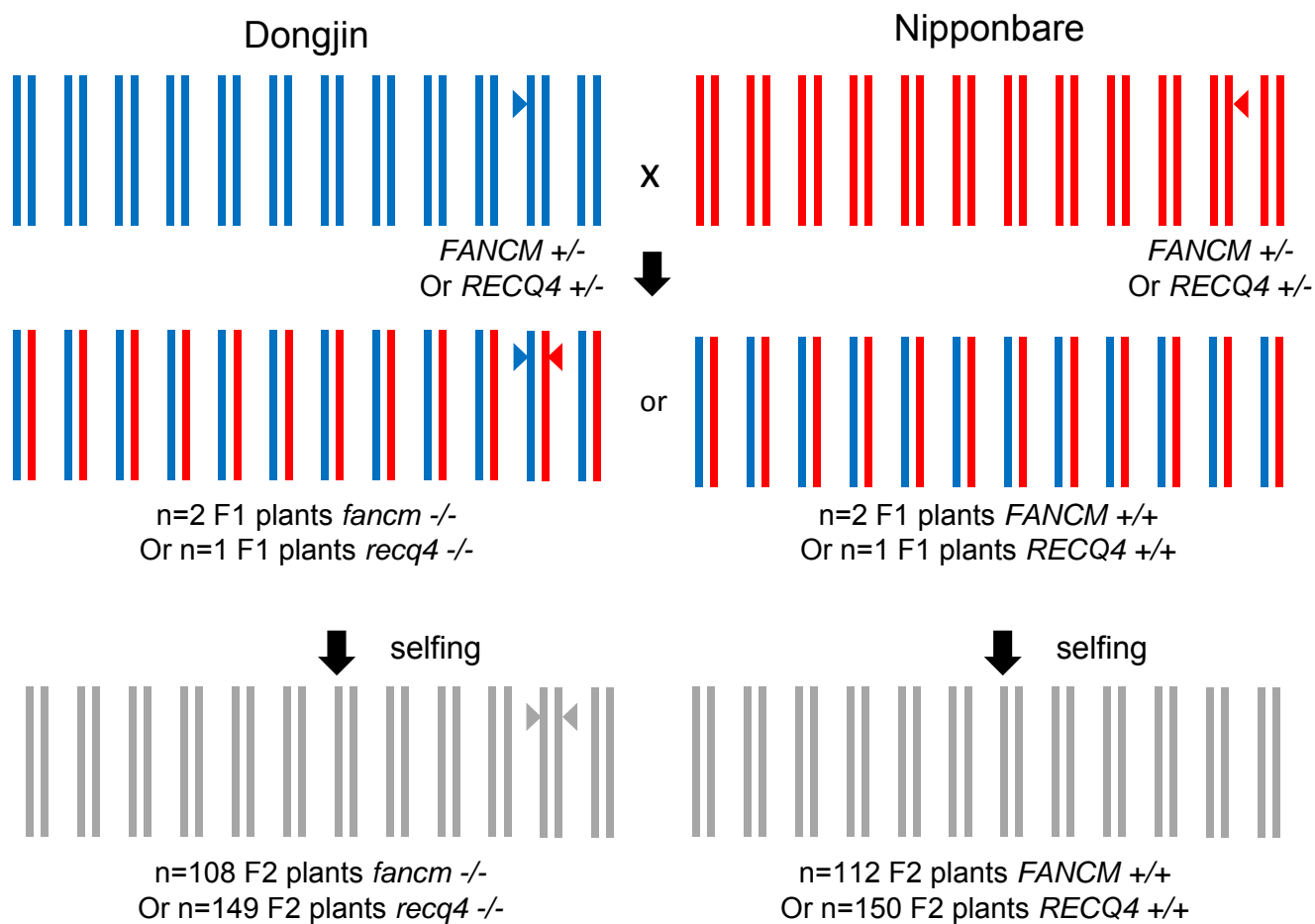


Figure S5. Experimental scheme for rice *fancm* or *recq4* Dongjin/Nipponbare hybrid populations.

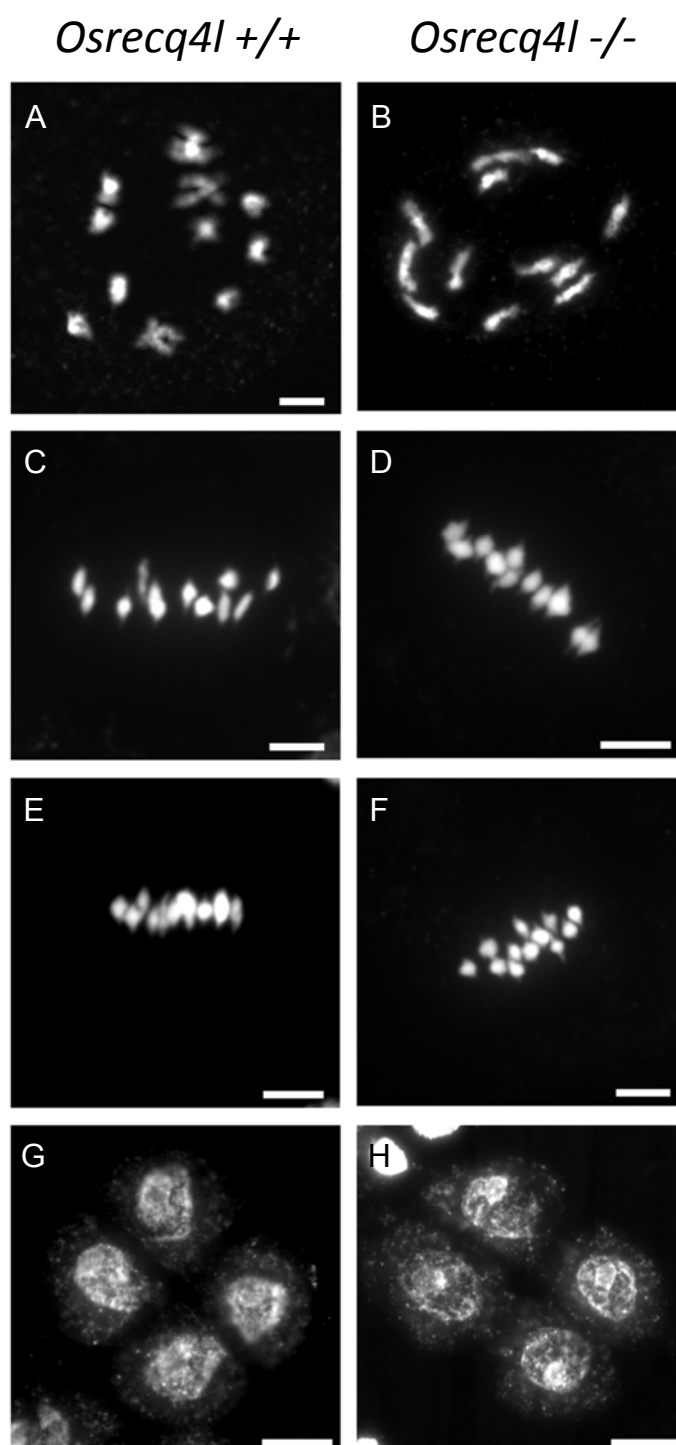


Figure S6. Male meiosis in *Osrecq4l* $-/-$

(A-B) Diplotene, the 12 pairs of chromosome are connected by chiasma. (C-D) Metaphase I with 12 aligned bivalents. (E-F) Metaphase II with 12 pairs of chromatids. (G-H) Telophase II. Male meiotic chromosome spreads were performed as previously described in [17]. Scale bar = 5 μ m.

Pea

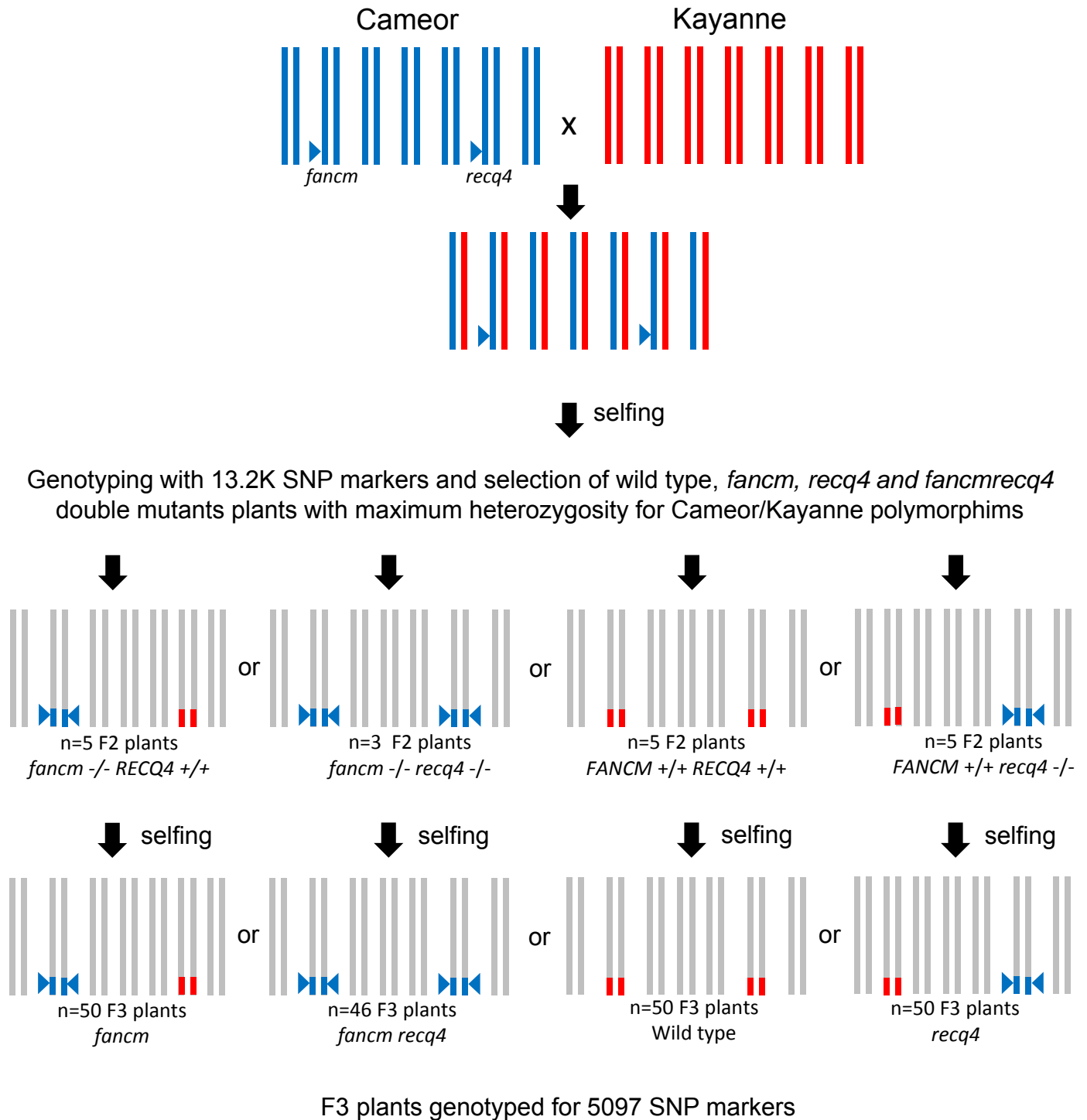


Figure S7. Experimental scheme for Pea *recq4 fancm* Cameor/Kayanne hybrid population

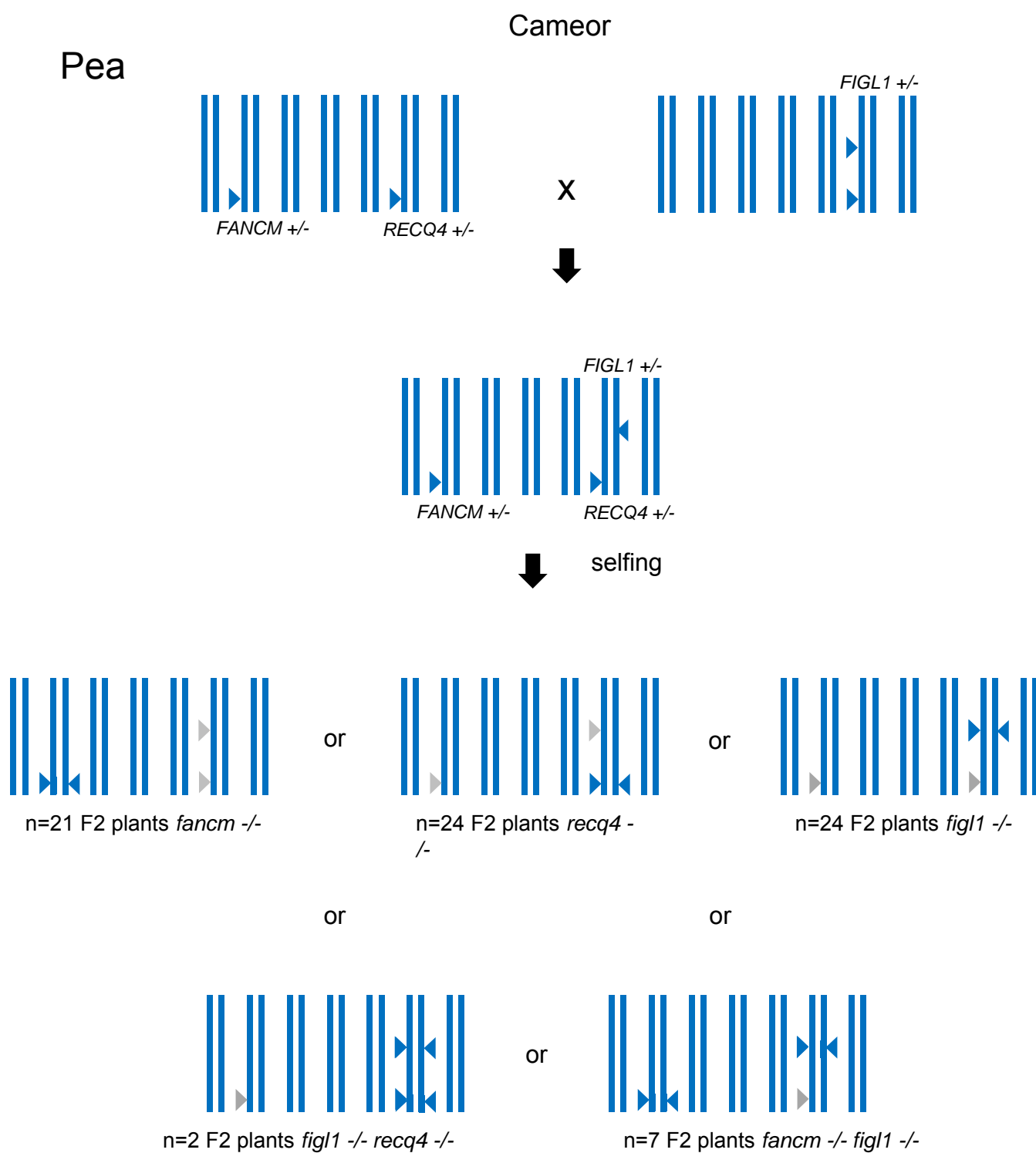


Figure S8. Crossing scheme for the Pea *recq4 fancm figl1* Cameor population.

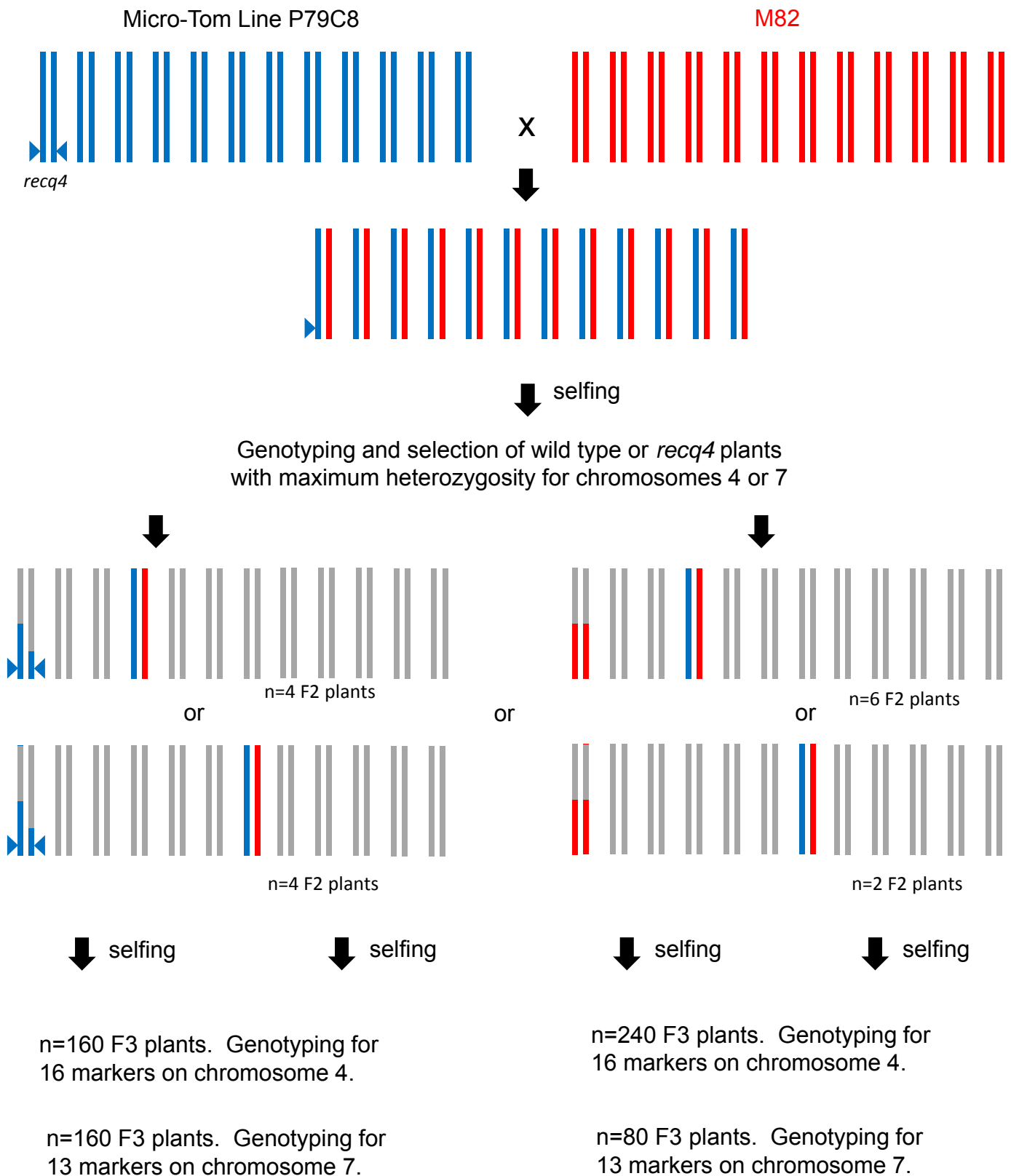


Figure S9. Experimental scheme for Tomato *recq4* Micro-Tome/M82 hybrid population

	Target	Primer name	Sequence (5' to 3')	PCR amplicon size (bp)
OsFANCM genotyping primers	<i>Osfancm-1</i>	AQS-BP	GTCTGGACCGATGGCTGTGTAGAAG	Mutant PCR = 798
		AQS-LP	AGGTATCCTTGGGGAGTTGG	Wild type PCR = 593
		AQS-RP	TGTAACATCCGATCAGTGTGC	
	<i>Osfancm-2</i>	A4-BP	TCGTAAAACCTGCCTGGCACAGC	Mutant PCR = 758
		A4-LP	CAACGTATGGGAAGGACTGG	Wild type PCR = 1094
		A4-RP	GGTGAAGAAGAACCAACCA	
OsRECQ4 genotyping primers	<i>Osrecq4-3</i>	AUF-BP	TCGTAAAACCTGCCTGGCACAGC	Mutant PCR = 690
		AUF-LP	TCGATGAATCGTCAGTTCCA	Wild type PCR = 1013
		AUF-RP	ACATGCGCTACGGGAECTAT	
	<i>Osrecq4-1</i>	3503-BP	ACGTCCGCAATGTGTTATTAA	Mutant PCR = 529
		3503-LP	GCTACATTTTGGAACGGAGGT	Wild type PCR = 1087
		3503-RP	TGGAGTGGTCAGAACAGCAG	

Table S1 : Primer sequences used for genotyping rice mutants and SNP position in the rice genome (MSU v7.0).

Wild type PCR was done with LP (Left primer) and RP (Right primer) primers; Mutant PCR with BP (Backbone primer) and RP primers.