

1 **Towards a catalog of pome tree architecture genes: the draft**

2 **‘d’Anjou’ genome (*Pyrus communis* L.)**

3 *Innovating towards architecture genes in pear*

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22

23 **Abstract**

24 The rapid development of sequencing technologies has led to a deeper understanding of
25 horticultural plant genomes. However, experimental evidence connecting genes to important
26 agronomic traits is still lacking in most non-model organisms. For instance, the genetic
27 mechanisms underlying plant architecture are poorly understood in pome fruit trees, creating a
28 major hurdle in developing new cultivars with desirable architecture, such as dwarfing rootstocks
29 in European pear (*Pyrus communis*). Further, the quality and content of genomes vary widely.
30 Therefore, it can be challenging to curate a list of genes with high-confidence gene models
31 across reference genomes. This is often an important first step towards identifying key genetic
32 factors for important traits. Here we present a draft genome of *P. communis* ‘d’Anjou’ and an
33 improved assembly of the latest *P. communis* ‘Bartlett’ genome. To study gene families involved
34 in tree architecture in European pear and other rosaceous species, we developed a workflow
35 using a collection of bioinformatic tools towards curation of gene families of interest across
36 genomes. This lays the groundwork for future functional studies in pear tree architecture.
37 Importantly, our workflow can be easily adopted for other plant genomes and gene families of
38 interest.

39

40 **Introduction**

41 Advancements in plant genome sequencing and assembly have vigorously promoted research in
42 non-model organisms. In horticultural species, new genome sequences are being released every
43 month¹⁻⁶. These genomes have broadened our understanding of targeted cultivars and provided
44 fundamental genomic resources for molecular breeding and more in-depth studies of
45 economically important crop traits such as those involved in plant architecture. Although many

46 gene families have been identified as important for architectural traits, such as dwarfing,
47 weeping, and columnar growth⁷, the study of these genes and their functionality in new species is
48 still hampered by inaccurate information about their gene models or domain structures, and the
49 frequent lack of 1:1 orthology between related genes of different study species. Sequencing and
50 annotating a diversity of related genomes are crucial steps for obtaining this level of information.
51
52 Crops, most of which have gone through more than ten thousand years of domestication to meet
53 human requirements, have a wide diversity in forms, sometimes even within the same species⁸.
54 One such example is in the *Brassica* species, where *B. rapa* encompasses morphologically
55 diverse vegetables such as Chinese cabbage, turnips, and mizuna; and cabbage, stem kale, and
56 Brussels sprouts are the same biological species, *B. oleracea*. Therefore, a single reference
57 genome does not represent the complex genome landscape, or pan-genome, for a single crop
58 species. To understand the genetic basis of the diverse *Brassica* morphotypes, many attempts
59 have been made to explore the genomes of *Brassica*⁸⁻¹². In one of those attempts, genomes from
60 199 *B. rapa* and 119 *B. oleracea* accessions were sequenced and analyzed using a comparative
61 genomic framework^{10,12}. Genomic selection signals and candidate genes were identified for traits
62 associated with leaf-heading and tuber-forming morphotypes. Compared to *Brassica*, pome fruits
63 may not appear to have as much diversity in their vegetative appearance, but they do have great
64 diversity in terms of fruit quality, rootstock growth and performance, and post-harvest
65 physiology. However, genome studies and pan-genome scale investigations in pome fruits are
66 still in their infancy. In cultivated apple (*Malus domestica*), genomes of three different
67 cultivars¹³⁻¹⁶ have been published, providing resources to study: 1) small (SNPs and small
68 InDels) and large scale (chromosome rearrangements) differences that can help explain cultivar

69 diversity, and 2) gene content differences that may contribute to cultivar specific traits. However,
70 genomic resources for European pear (*Pyrus communis*) cultivars are limited to just two
71 published genomes^{17,18} from a single cultivar, ‘Bartlett’. More European pear genomes will
72 afford new perspectives that help us understand shared and unique traits for important cultivars
73 in *Pyrus*, as well as other Rosaceae.

74

75 Besides understanding large scale genomic characteristics, new genomes also provide rich
76 resources for reverse genetic studies^{19,20}. To obtain the actual sequence of a target gene, reverse
77 genetic approaches in the pre-genome era relied on sequence and domain homology and
78 technologies such as RACE PCR²¹, which could be challenging and time consuming.

79 Alternatively, in species with high-quality reference genomes, the annotation is generally
80 considered to contain all the genes and target genes could ideally be identified with a sequence
81 similarity search (*i.e.*, BLAST). However, reports of annotation errors, such as imperfect gene
82 models and missing functional genes are very common^{22,23,24}. Another complicating factor is that
83 duplication events (*i.e.*, whole genome duplication, regional tandem duplication) and polyploidy
84 occur in the majority of flowering plants, including most crop species, posing substantial
85 challenges to genome assembly and annotation²⁵. Moreover, instances of neofunctionalization
86 and subfunctionalization occur frequently following duplication events²⁶, which sometimes will
87 result in large and complex gene families^{27,28}. Therefore, a one-to-one relationship between a
88 gene in a model organism and its ortholog in other plant species, or even between closely related
89 species and varieties, is rare²⁹. Without understanding the orthology and paralogy between
90 members of a given gene family, it is difficult to translate knowledge of a gene in a model
91 organism to another species of interest.

92

93 In the present study, we assembled a draft genome for the European pear cultivar ‘d’Anjou’,
94 improved the current ‘Bartlett’ assembly (*i.e.*, Bartlett.DH_V2), and developed a workflow that
95 allows highly efficient target gene identification in any plant genome of interest. We used our
96 workflow to curate and improve gene models for architecture-related genes from both the
97 polished Bartlett.DH_v2 and the d’Anjou genomes. Importantly, we recovered many genes that
98 were missing from gene families of interest (50 genes in the cultivar ‘Bartlett’) and corrected
99 errors in others across the genus *Pyrus*. This work demonstrates that the integration of
100 comparative genomics and phylogenomics can facilitate and enhance gene annotation, and thus
101 gene discovery, in important plant reference genomes.

102

103 **Results**

104 **The draft d’Anjou genome**

105 *Genome assembly*

106 We generated approximately 134 million paired-end reads from Illumina HiSeq and a total of
107 1,054,992 PacBio continuous long reads (CLR) with a read length N50 of 20 Kb, providing an
108 estimated 67-fold and 21-fold coverage respectively of the expected 600 Mb *Pyrus communis*
109 genome¹⁸. Additionally, approximately 468 million 2 x 150 bp paired reads (~234-fold coverage)
110 with an estimated mean molecule length (linked-reads) of 20 kb were generated using 10x
111 Genomics Chromium Technology (Supplementary Table 1). The final meta-assembly, generated
112 with a combination of the three datasets, contains 5,800 scaffolds with a N50 of 358 Kb (Table
113 1). The cleaned contigs and scaffolds were ordered and oriented into 17 pseudochromosomes
114 guided by the reference genome, *Pyrus communis* ‘Bartlett.DH_v2’¹⁷.

115

116 Next, we compared the d'Anjou meta-assembly to two published reference assemblies of
117 Bartlett^{17,18} to assess assembly contiguity, completeness, and structural accuracy. The
118 Benchmarking Universal Single-Copy Ortholog (BUSCO)³⁰ analysis showed that the d'Anjou
119 genome captured 96.6% complete genes in the Embryophyta gene sets, comparable to the
120 reference genomes (Table 1, Supplementary Table 2). Furthermore, synteny comparisons
121 between the draft d'Anjou genome and the reference Bartlett.DH_v2 genome showed high
122 collinearities at both whole-genome and chromosomal levels (Fig. 1a and Supplementary Fig. 1).

123

124 *Annotation*

125 Combining information such as *de novo* transcriptome assembly, homologous proteins of closely
126 related species, and protein-coding gene annotations from the two Bartlett genomes, we
127 identified a total of 45,981 protein coding genes in d'Anjou (Table 1). Of those putative genes
128 76.63% were annotated with functional domains from Pfam³¹ and the remaining are supported by
129 annotation evidence, primarily d'Anjou RNA-Seq reconstructed transcript³². These results
130 indicate that we captured a large majority of the gene space in the d'Anjou genome. This affords
131 a range of analyses including gene and gene family characterization, plus global-scale
132 comparisons with other Rosaceae including the 'Bartlett' cultivar.

133

134 **Comparison among three European pear genomes**

135 To study the shared and genotype-specific genes among the three European pear genomes, we
136 constructed 25,511 protein clusters, comprising 77.71% of all the genes. While numbers of
137 predicted genes from the Bartlett_v1 and d'Anjou genomes may be overestimated due to the

138 presence of alternative haplotype segments in the assembly caused by high heterozygosity¹⁷, this
139 should have very little effect on orthogroup circumscription. Further, the process of creating a
140 double haploid reduces genome heterozygosity, but should retain estimates of orthogroup
141 content. Hence, we formulated the following hypotheses: 1) a large majority of gene families are
142 shared by all three genotypes; 2) few genotype-specific gene families are present in each
143 genome; 3) the commercial ‘Bartlett’ genotype and the double haploid “Bartlett’ genotype
144 (roughly version 1.0 and 2.0 of this genome, respectively) should have virtually identical gene
145 family circumscription; and 4) we should detect very few gene families that are unique to either
146 ‘Bartlett’ genome and shared with ‘d’Anjou’. The protein clustering analysis results (Table 1,
147 Fig. 1b) support our hypotheses 1 and 2: 65.60% of the orthogroups contain genes from all three
148 genotypes and only 0.12% of the orthogroups are species-specific. However, among the 8,744
149 orthogroups containing genes from two genotypes, more than half (55.11%) are shared between
150 d’Anjou and Bartlett_v1, 18.10% are shared by d’Anjou_v1 and Bartlett.DH_v2, and only
151 26.80% are shared between the two Bartlett genomes, which does not support hypotheses 3 and
152 4.

153
154 To better understand why these hypotheses lacked support, we took a broader look at gene
155 family content by comparing a collection of Rosaceae genomes, including the pear genomes in
156 question. We assigned all the predicted protein coding genes from genomes of interest^{13–15,17,18} to
157 orthogroups constructed with a 26-genome scaffold, covering most of the major lineages of land
158 plants (supplementary Fig. 2). Out of the 18,110 orthogroups from this database, *Prunus persica*,
159 a rosaceous species included in the genome scaffold, has representative genes in 10,290
160 orthogroups. Genes from most apple and pear genomes (Bartlett_v1, d’Anjou_v1, *Malus*

161 *domestica* HFTH_v1.0, *M. domestica* GDDH13_v1.1, *M. domestica* Gala_v1.0, *M.*
162 *sieversii*_v1.0, *M. sylvestris*_v1.0) are present in more than 9,800 orthogroups, however, genes
163 from Bartlett.DH_v2 were only found in 9,688 orthogroups (Table 1 & Supplementary Table 3).
164 These results suggest there are many genes not annotated in the Bartlett.DH_v2 genome.

165

166 **Genome-Wide identification of selected architecture genes**

167 *A selection of architecture genes*

168 With this new comparative genomic information, our next steps were two-fold: first, to leverage
169 information from the three European pear genomes and other available Rosaceae genomes, to
170 identify and improve a set of tree architecture-related gene models of interest, and second, to use
171 these architecture gene families as a test case to investigate potential issues in the Bartlett.DH_v2
172 genome. Many aspects of tree architecture are important for improving pear growth and
173 maintenance, harvest, ripening, tree size and orchard modernization, disease resistance, and soil
174 microbiome interaction. Traits of interest include dwarfing and dwarfism, root system
175 architecture traits, and branching and branch growth. We selected key gene families known to be
176 involved (Table 2)³³⁻⁶⁴, particularly those that have been previously shown to influence
177 architectural traits in fruit trees. The identification of genes within these families, as well as their
178 genomic locations, correct gene models, and domain conservation, is an important early step in
179 testing and understanding their relationships and functions.

180

181 *Overview of the gene identification workflow*

182 Here, we developed a high throughput workflow (Fig. 2), leveraging a subset of the best
183 Rosaceae plant genomes, and a phylogenomic perspective to efficiently and accurately generate

184 lists of genes in gene families of interest and phylogenetic relationships of genes from different
185 plant lineages. Our workflow, consisting of three main steps, implemented various functions
186 from PlantTribes⁶⁵ (<https://github.com/dePamphilis/PlantTribes>) and other software^{66,67} for
187 targeted gene annotation.

188

189 *Step 1 - An initial gene list and preliminary phylogenies*

190 In Step 1, representative plant architecture genes obtained from the literature were assigned into
191 orthogroups based on sequence similarity, giving us 22 orthogroups of interest (Supplementary
192 Tables 4-5. Note that OG12636 is a monocot-specific orthogroup, thus not included in the
193 downstream analysis of this section). In parallel, we classified all the genes annotated from 14
194 Rosaceae genomes (Supplementary Fig. 2) into the same database. Next, Rosaceae genes
195 assigned into the 21 orthogroups were integrated with sequences from the 26 scaffolding species
196 for multiple sequence alignments, which were used to infer phylogeny. At the end of this step,
197 we obtained our initial list of genes in each orthogroup and the phylogenetic relationships of
198 each gene family.

199

200 After examining the 21 orthogroups, we identified 64, 105, 94, and 53 genes from *Prunus*
201 *persica*, Gala_v1, d'Anjou, and Bartlett.DH_v2, respectively. A whole genome duplication
202 (WGD) event occurred in the common ancestor of *Malus* and *Pyrus*¹⁴, but was not shared with
203 *Prunus*. Therefore, we expect to see an approximate 1:2 ratio in gene numbers in most cases,
204 which explains fewer genes in *Prunus* compared to Gala_v1 and d'Anjou. However, the low
205 gene count in Bartlett.DH_v2 was unexpected. For instance, we observed a clade within a PIN
206 orthogroup (OG1145) comprised of short *PIN* genes³⁸, which seemed to lack genes from the

207 Bartlett.DH_v2 genome altogether (Fig. 3a). One gene copy is found in *Prunus* and Rosoideae
208 species, and two copies are found in most of the Maleae species, but none were identified in
209 Bartlett.DH_v2. In addition, in the four genomes mentioned above, we found a number of
210 problematic genes (Supplementary Table 6), for example genes that appeared shorter than all
211 other orthologs or contained unexpected indels likely due to assembly or annotation errors.

212

213 *Step 2 and 3 - Iterative reannotation of problematic gene models*

214 Inaccurate and missing gene models are common in any genome, especially in the early
215 annotation versions^{23,24}. In model organisms, such as human, mouse
216 (<https://www.encodegenes.org/>), and *Arabidopsis* (<https://www.arabidopsis.org/>), gene
217 annotations are continuously being improved using experimental evidence, improved data types
218 (e.g. full-length RNA molecule sequencing), and both manual and computational curation.
219 Building a better genome assembly is another way to detect additional genes. For instance, the
220 BUSCO completeness score increased from 86.7% in the initial ‘Golden Delicious’ apple
221 genome¹⁶ to 94.9% in the higher-quality GDDH13 genome¹⁵, indicating that the latter genome
222 captured approximately 120 more conserved single-copy genes. Hence, we hypothesized that the
223 potentially missing and problematic gene models we observed in the two European pears could
224 be improved by: 1) using additional gene annotation approaches; and 2) searching against
225 improved genome assemblies.

226

227 To test whether further gene annotation would improve problematic gene models, we moved
228 forward to Step 2 of our workflow, using results from Step 1 as inputs. For each orthogroup
229 containing problematic European pear genes (Supplementary Table 6), we used a subset of high-

230 quality gene models from Rosids identified in Step 1 as inputs and re-annotated these gene
231 families in the two pear genomes. After using a combination of annotation softwares and manual
232 curation, we found a total of 98 genes from the d'Anjou genome, and reduced the number of
233 problematic or incomplete genes from 34 to 3. In Bartlett.DH_v2, we identified 20 complete
234 genes that were not annotated in the original genome and improved the sequences of 7
235 previously problematic genes, however, the total number of the selected architecture genes (73
236 genes among which 15 were problematic or incomplete) was still notably lower than that of
237 d'Anjou (98 with 3 incomplete genes) or Gala (105 with 15 being incomplete, see
238 Supplementary Table 6). In Step 3, which involves iterative steps of phylogenetic analysis and
239 targeted gene re-annotation, we added additional information such as the improved d'Anjou
240 genes and RNA-seq datasets as new resources to annotate Bartlett.DH_v2 genes, but found no
241 improvements in identifying unannotated genes or improving problematic models.

242

243 Results gathered after the first iteration of Step 3 supported our hypothesis that extra annotation
244 steps could help improve imperfect gene models and identify missing genes in the two targeted
245 European pear genomes. However, there were still about 30 genes potentially missing in
246 Bartlett.DH_v2, which led us to test whether polishing the genome assembly would further
247 improve problematic or missing gene models.

248

249 *Step 3 - Adding Bartlett.DH_v2 genome polishing*

250 The quality of genome assembly is affected by many factors, including sequencing depth, contig
251 contiguity, and post-assembly polishing. Attempts to improve a presumably high-quality genome
252 are time consuming, and may prove useless if the genome is already in good condition. To

253 initially determine whether polishing the genome assembly would be useful, we first investigated
254 the orthogroups with problematic Bartlett.DH_v2 genes to seek for evidence of assembly derived
255 annotation issues. Indeed, in most cases where we failed to annotate a gene from presumably the
256 correct genomic region, we observed unexpected indels while comparing the Bartlett.DH_v2
257 genome assembly to other pear genomes (Supplementary Fig. 3 and Supplementary Table 7).
258 Unexpected indels in the Bartlett.DH_v2 genome were associated with incorrect gene models as
259 well. For example, Fig. 3b shows a subset of amino acid sequence alignments for a specific
260 member (*Pyrco_BartlettDH_13g21160*) of a PIN orthogroup (OG438) comprised of the long
261 *PIN* genes³⁸, in which the Bartlett.DH_v2 gene model shared low sequence identity with
262 orthologs from other Maleae species and *Prunus*. To validate the identity of the problematic gene
263 models, we leveraged RNAseq data from various resources⁶⁸⁻⁷³ and mapped them to the
264 Bartlett.DH_v2 gene models. In most cases where a conflict was present between the pear
265 consensus, for a given gene of interest, and the Bartlett.DH_v2 gene model, the reads supported
266 the consensus (Fig. 3c). The frequent occurrence of truncated and missing genes in the
267 Bartlett.DH_v2 genome may be caused by assembly errors (*e.g.*, base call errors, adapter
268 contamination) that create erroneous open reading frames. This observation provided us with the
269 first piece of evidence that the differences in gene family content observed in the Bartlett.DH_v2
270 genome may not only be caused by misannotations, but also assembly issues.

271
272 To further test whether improvement to the genome assembly would allow us to capture the
273 problematic and missing genes, we polished the Bartlett.DH_v2 genome with Illumina reads
274 from the original publication¹⁷. We identified 98.40% complete BUSCOs in the polished
275 genome assembly, a 1.90% increase compared to the original assembly (Supplementary Table 2).

276 Using the polished genome, we reiterated Step 3 of our workflow and annotated a total of 103
277 genes in our gene families of interest, with only two gene models being incomplete
278 (Supplementary Table 6). This new result doubled the number of genes we identified from the
279 original genome annotation and brought the expected gene number into parity with other pome
280 fruit genomes. This supports our hypothesis that genes were missing due to methodological
281 reasons, and in this case, due to assembly errors.

282

283 **Curation of a challenging gene family: the IGT family**

284 Some gene families are more complex than others. For example, it is more difficult to study the
285 evolution of resistance (R) genes than most BUSCO genes because the former is comprised of
286 fast-evolving multigene families while the latter are universally conserved single-copy gene
287 families. Within the architecture gene families we studied, the IGT family is more challenging
288 than many others because members of this family have relatively low levels of sequence
289 conservation outside of a few conserved domains⁷⁴. Previous reports identified four major clades
290 (LAZY1-like, DRO1-like, TAC1-like, and LAZY5-like) in this gene family³⁴. Study of LAZY1
291 in model species identified 5 conserved regions⁷⁴ (Fig 4c). The same domains are also present in
292 other LAZY1-like and DRO1-like proteins and the first 4 domains are found in TAC1-like
293 proteins across land plants⁷⁵. LAZY5-like, the function of which is largely unknown, has only
294 domains I and V. Early research of the *TAC1-like* and *LAZY1-like* IGT genes identified these
295 genes as grass-specific^{76,77}, as BLAST searches failed to find homologs in other plant lineages.

296

297 Using Arabidopsis and rice *IGT* genes as queries, our workflow identified five orthogroups
298 (Supplementary Table 4), containing all the pre-characterized *IGT* genes in angiosperms. The

299 phylogeny constructed with these five orthogroups largely supported previous classification of
300 the four clades³⁴, and provided more information regarding the evolutionary history of this gene
301 family (Fig. 4a and Supplementary Fig. 4). The TAC1-like clade, which is sister to the others, is
302 divided into two monophyletic groups; one contains only monocots while the other has
303 representatives from all the other angiosperm lineages. The LAZY1-like and LAZY5-like clades
304 form one large monophyletic group, which is sister to the DRO1-like clade. Within Rosaceae, a
305 near 1:2 ratio was expected between peach and pear due to the WGD in the common ancestor of
306 the Maleaes. Compared to the six known peach *IGT* genes³⁴, we found 11 orthologs in
307 Bartlett.DH_v2 (including 1 short gene, *Pycro_BartlettDH_LAZY.Chr10*, caused by an
308 unexpected premature stop codon) and 9 in d'Anjou (*Pycro_Danjou_DRO.Chr2* and
309 *Pycro_Danjou_LAZY.Chr10* failed to be annotated due to missing information in the genome).
310 The resulting phylogeny (Fig. 4a) shows that we have now identified most of the expected *IGT*
311 genes in European pears.

312

313 Besides low sequence similarity, *IGT* genes also have unique intron-exon arrangements, which
314 are conserved across Arabidopsis and a few other plant species^{34,74,78}. These genes all contain 5
315 exons, but unlike most genes, the first exon only comprises six nucleotides and the last exon
316 contains ~20 nucleotides. Annotation of short exons, especially when transcriptome evidence is
317 limited, can be very challenging and skipping such exons could cause problems in gene
318 discovery⁷⁹⁻⁸¹. For instance, the annotation of *AtAPC11* (*At3g05870*) was inaccurate until Guo
319 and Liu identified a single-nucleotide exon in this gene⁸⁰.

320

321 To determine whether we captured the correct *IGT* gene models in the targeted genomes, we
322 investigated the protein sequence alignments and gene features. In the original annotation, only
323 three gene models (*Pyrco_BartlettDH_16g10510*, *Pyrco_BartlettDH_07g15250*,
324 *Pyrco_DAnjou_Chr7v0.1_17442.1*) have the correct intron-exon combination and the expected
325 domains. In the iterative re-annotation steps of our workflow, we identified 6 additional accurate
326 gene models leveraging sequence orthology and transcriptome evidence⁶⁸⁻⁷³. We further
327 investigated all the sequences we identified as *IGT* genes, seeking the presence or absence of the
328 expected domain features. However, even among gene models from the best annotated genomes
329 used to construct the 26Gv2.0 database, only 45.16% (56/124) have the expected domain
330 features (Indicated with an * next to gene names in Fig. 4a) (LAZY5-like was not taken into
331 consideration due to its unique structure). In most cases, although the signature *IGT* domain (II)
332 is correctly identified in the genes, domains I and V are usually missing or incorrect, likely due
333 to mis-annotation of the first and last short exons. In Rosaceae, besides *Bartlett.DH_v2* and
334 *d'Anjou*, 34.38% (33/96) had the expected domains (Fig. 4a). This finding motivated us to
335 manually investigate the targeted genomes to annotate the *IGT* genes. Using the correct gene
336 models as reference, plus a careful manual curation, we were able to annotate 19 complete gene
337 models of 20 expected *IGT* genes from the two targeted pear genomes (Figs. 4b and 4c).

338

339 **Discussion**

340 A second European pear cultivar genome from 'd'Anjou' provided additional insights into gene
341 families across Rosaceae. By leveraging perspectives from comparative genomics and
342 phylogenomics, we developed a high-throughput workflow using a collection of bioinformatic

343 tools that takes a list of genes of interest from the literature and genomes of interest as input, and
344 produces a curated list of the targeted genes in the query genomes.

345

346 In the case study presented here, candidate genes from 16 plant architecture-related gene families
347 were identified from 14 Rosaceae genomes. The study of gene families consists primarily of two
348 initial parts: first, identification of all the members in these families, and second, investigation of
349 their phylogenetic relationships. Many attempts⁸²⁻⁸⁴ to identify genes of interest from a genome
350 have relied solely on a BLAST search querying a homolog from a model organism, which may
351 be distantly related. However, such a method is insufficient in identifying all members of a large
352 complex gene family or a fast-evolving and highly-divergent family, such as the *IGT* genes.

353 They may also incorrectly include genes in a gene family based only on one or a few highly
354 conserved regions that are insufficient for gene family membership. Compared to a BLAST-only
355 approach, the gene classification process in our workflow used a combination of BLAST and
356 HMMER search of an objectively pre-classified gene family scaffold, which provides a better
357 result by taking into consideration both sensitivity and specificity⁶⁵. This allowed us to
358 efficiently identify even very challenging genes. Moreover, phylogenetic relationships revealed
359 by a small number of taxa, for instance using only one species of interest and one model
360 organism, can be inaccurate. For example, in our phylogenetic analysis with rich taxon sampling,
361 *PIN5-1* and *PIN5-2* from *Pyrus bretschneideri* are sisters to all other *PINs* (Supplementary Fig.
362 5), challenging the phylogenetic relationship inferred with *PINs* only from *P. bretschneideri* and
363 *Arabidopsis thaliana*⁶¹.

364

365 The iterative quality control steps in the workflow helped identify problems that existed in
366 certain gene models and provided hints about where to make targeted improvements to important
367 *Pyrus* genomic resources. The highly contiguous assembly of Bartlett.DH_v2 provided a
368 valuable reference to anchor the shorter scaffolds from d'Anjou, which is essential for a good
369 annotation. On the other hand, the perspective afforded by the d'Anjou genome led us to
370 examine the Bartlett.DH_v2 genome assembly further. We developed and tested hypotheses
371 regarding unexpected gene annotation patterns in the two targeted European pear genomes
372 among various Maleae species and cultivars. This led to a polished assembly and improved
373 annotations that allowed us to curate a high confidence list of candidate genes and gene models
374 for downstream analyses. By adding targeted iterations of genome assembly and annotation, we
375 now have a better starting point for reverse genetic analyses and understanding functionality of
376 architecture-related genes in pears.

377

378 The challenges we encountered as we laid the groundwork for reverse genetics studies to
379 understand pear architecture genes, and the approaches we took to evaluate and tackle these
380 challenges, reinforce the idea that genome assembly and annotation are iterative processes. We
381 found that relating gene accession IDs and inconsistent gene names back to gene sequences in
382 various databases was often difficult and time consuming. Objective, global-scale gene
383 classification, as we used here via PlantTribes^{65,85}, can help researchers work across genomes
384 and among various genome resources. Further, guidance from consortia such as AgBioData⁸⁶ is
385 helping facilitate work such as we have described here that includes the acquisition and analysis
386 of genome-scale data. Our starting point for understanding putative architecture genes in pear
387 was with genes of interest from several plant species - an approach that many researchers will

388 find familiar. With genes of interest in hand, our workflow provides a comparative genome
389 approach to efficiently identify, investigate, and then improve and/or validate genes of interest
390 across genomes and genome resources.

391

392 **Materials and Methods**

393 **Plant materials and sequencing**

394 The 'd'Anjou' plants were purchased from Van Well's nursery in East Wenatchee, WA, USA
395 and grown in the USDA ARS greenhouse #6 at Wenatchee, WA, USA. Fresh leaves (~1.5g)
396 from one 'd'Anjou' plant were flash frozen and used for DNA extraction. A CTAB isolation
397 protocol⁸⁷ was used to generate high-molecular-weight genomic DNA with the following
398 modifications: the extraction was performed large scale in 100 ml of extraction buffer in a 250
399 ml Nalgene centrifuge bottle; the isopropanol precipitation was performed at room temperature
400 (~ 5 minutes) followed immediately by centrifugation; after a 15-minute incubation in the first
401 pellet wash solution, the pellet was transferred to a 50 ml centrifugation tube via sterile glass
402 hook before performing the second pellet wash; following the second pellet wash, centrifugation
403 and air drying, the pellet was resuspended in 2 ml TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0)
404 and allowed to resuspend at 4 °C overnight. The concentration of the DNA was measured by a
405 Qubit 2.0 fluorometer (Invitrogen) and 50 ug DNA was digested with RNase A (Qiagen, final
406 concentration 10 ug/ml, 37 °C for 30 minutes) and then further cleaned up using the PacBio
407 recommended, user-shared gDNA clean-up protocol
408 (<https://www.pacb.com/search/?q=user+shared+protocols>) performed at large-scale with the
409 DNA sample brought up to 2 ml with TE and all other volumes scaled up accordingly. The final
410 pellet was resuspended in 100 ul TE. The final DNA concentration was measured by Qubit

411 fluorometer, and 500 ng was loaded onto a PFG (Bio-Rad CHEF) to check the size range. The
412 DNA ranged in size from 15 Kb to 100 Kb with a mean fragment size around 50 Kb. The purity
413 of the DNA as measured by the NanoDrop spectrophotometer (ThermoFisher) was 260/280 nm:
414 1.91; 260/230 nm: 2.51. Cleaned-up gDNA was sent to the Penn State Genomics Core facility
415 (University Park, PA, USA) for Pacbio and Illumina library construction and sequencing. A total
416 of 10 ug gDNA was used to construct PacBio SMRTbell libraries and sequenced on a PacBio
417 Sequel system. A small subset of the same gDNA was used to make Illumina TruSeq library and
418 was sequenced on an Illumina HiSeq 2500 platform. In addition, 4 ug of the same gDNA was
419 sent to the DNA technologies and Expression Analysis Core Laboratory at UC Davis (Davis,
420 CA, USA) to construct an Illumina 10X Chromium library, which was sequenced on an Illumina
421 NovaSeq 6000 sequencer.

422

423 **Genome assembly and post-assembly processing**

424 To create the initial backbone assembly of d'Anjou, Canu assembler v2.1.1⁸⁸ was used to correct
425 and trim PacBio continuous long reads (CLR) followed by a hybrid assembly of Illumina short
426 reads and PacBio CLR with MaSuRCA assembler v3.3.2⁸⁹. Next, Supernova v2.1.1, the 10x
427 Genomics *de novo* assembler⁹⁰, was used to assemble linked-reads at five different raw read
428 coverage depths of approximately 50x, 59x, 67x, 78x, and 83x based on the kmer estimated
429 genome size, and the resulting phased assembly graph was translated to produce two parallel
430 pseudo-haplotype sequence representations of the genome. The Supernova assembler can only
431 handle raw data between 30- to 85-fold coverage of the estimated genome size. Therefore, the
432 multi-coverage assemblies provide an opportunity to capture most of the genome represented in
433 the ~234-fold coverage sequenced 10x Chromium read data. One of the pseudo-haplotypes at

434 each of the five coverages was utilized for subsequent meta-assembly construction to improve
435 the backbone assembly using a combination of assembly metrics, including 1) contig and
436 scaffold contiguity (L50), 2) completeness of annotated conserved land plants (embryophyta)
437 single-copy BUSCO genes³⁰, and 3) an assembly size closer to the expected d'Anjou haploid
438 genome size. The backbone assembly was incrementally improved by bridging gaps and joining
439 contigs with the Quickmerge program⁹¹ using contigs from the five primary Supernova
440 assemblies in decreasing order of assembly quality. The resulting meta-assembly at each
441 merging step was only retained if improvement in contiguity, completeness, and assembly size
442 was observed.

443

444 Next, the long-distance information of DNA molecules provided in linked-reads was used to
445 correct assembly errors introduced in the meta-assembly during both the *de novo* and merging
446 steps of the assembly process with Tigmint⁹² and ARCS⁹³ algorithms. Tigmint aligns linked
447 reads to an assembly to identify potential errors and breaks assembled sequences at the
448 boundaries of these errors. The assembly is then re-scaffolded into highly contiguous sequences
449 with ARCS utilizing the long-distance information contained in the linked reads. To further
450 improve the d'Anjou meta-assembly, trimmed paired-reads from both the short insert Illumina
451 and 10x Chromium libraries were utilized to iteratively fill gaps between contigs using GapFiller
452 v1.10⁹⁴, and correct base errors and local misassemblies with Pilon v1.23⁹⁵. The genome
453 assembly process is illustrated in Supplementary Fig. 6.

454

455 **Pseudomolecule construction**

456 Before constructing the d'Anjou nuclear chromosomal-scale pseudomolecules, extraneous DNA
457 sequences present in meta-assembly were identified and excluded (Supplementary Fig. 6).
458 Megablast searches with e-value < 1e-10 was performed against the NCBI nucleotide collection
459 database (nt), and then the best matching Megablast hits (max_target_seqs = 100) against the
460 NCBI taxonomy database were queried to determine their taxonomic attributions. Assembly
461 sequences with all their best-matching sequences not classified as embryophytes (land plants)
462 were considered contaminants and discarded. A second iteration of Megablast searches of all the
463 remaining sequences (embryophytes) was performed against the NCBI RefSeq plant organelles
464 database to identify chloroplast and mitochondrion sequences and assembly sequences with high
465 similarity (> 80% identity; > 50% coverage) to plant organelle sequences were discarded^{27,96}.
466 Finally, the remaining meta-assembly nuclear contigs and scaffolds were ordered and oriented
467 into chromosomal-scale pseudomolecules with RaGOO⁹⁷ using the European pear, *Pyrus*
468 *communis* Bartlett.DH_v2 genome¹⁷ reference chromosomes (Supplementary Fig. 6).

469

470 **Assembly validation**

471 Both the contig and scaffold assembly metrics were evaluated in addition to the completeness of
472 universally conserved single-copy genes using the BUSCO land plants (embryophyta)
473 benchmark gene set (Supplementary Table 8). Whole-genome synteny comparison between
474 Bartlett.DH_v2, the chromosome assembly of the Bartlett cultivar, and d'Anjou meta-assembly
475 were evaluated with D-GENIES⁹⁸ using repeat masked (<http://www.repeatmasker.org>) DNA
476 alignments performed with minimap2⁹⁹ for the whole genome and each of the 17 *Pyrus*
477 *communis* chromosomes as shown in Fig. 1 and Supplementary Fig. 6, respectively.

478

479 **Gene prediction**

480 To identify the regions of genomic DNA that encode genes, we first estimated the portion of
481 d'Anjou meta-assembly comprised of repetitive elements suitable for repeat masking prior to
482 protein-coding gene annotation following the protocol described by Campbell *et al* (2014)¹⁰⁰.
483 The meta-assembly was first searched using MITE-Hunter¹⁰¹ and LTRharvest/ LTRdigest^{102,103}
484 to collect consensus miniature inverted-repeat transposable elements (MITEs) and long terminal
485 repeat retrotransposons (LTRs) respectively. LTRs were filtered to remove false positives and
486 elements with nested insertions and used together with the MITEs to mask the genomes. The
487 unmasked regions of the genomes were then annotated with RepeatModeler
488 (<http://www.repeatmasker.org/RepeatModeler>) to predict additional *de novo* repetitive
489 sequences. All collected repetitive sequences were compared to a BLAST database of plant
490 proteins from SwissProt and RefSeq, and sequences with significant hits were excluded from the
491 repeat masking library.

492
493 Extensive extrinsic gene annotation homology evidence from RNA-seq and protein were
494 collected to supplement *ab initio* gene predictions. RNA-Seq evidence included Trinity¹⁰⁴ *de*
495 *novo* reconstructed transcripts from d'Anjou pear fruit peel and cortex tissues sampled at multiple
496 time points described in our previous study³². Protein homology evidence of closely related
497 species were collected from the Genome Database for Rosaceae (GDR), including *Malus*
498 *domestica*, *Prunus persica*, *Pyrus betulifolia*, *Pyrus communis* 'Bartlett', *Pyrus x bretschneideri*,
499 *Rosa chinensis*, and *Rubus occidentalis*¹⁰⁵. The plant model species, *Arabidopsis thaliana*¹⁰⁶, was
500 included as well.

501

502 Protein-coding gene annotations from the *Pyrus communis* reference genomes of Bartlett_v1 and
503 Bartlett.DH_v2 were separately transferred (liftovers) to pseudomolecules of d'Anjou meta-
504 assembly using the FLO (<https://github.com/wurmlab/flo>) pipeline based on the UCSC Genome
505 Browser Kent-Toolkit¹⁰⁷. Next, the MAKER annotation pipeline (release 3.01.02)¹⁰⁸ was used to
506 update the transferred annotations with evidence data and gene models predicted by *ab initio*
507 gene finders. Repetitive and low complexity regions of the pseudomolecules were first masked
508 with RepeatMasker in MAKER using the previously described d'Anjou-specific repeat library.
509 MAKER updated transferred annotations with evidence data and predicted additional annotations
510 with Augustus^{109,110} and SNAP¹¹¹ using the d'Anjou training set where evidence suggests a gene.
511 Only predicted gene models supported by annotation evidence, encode a Pfam domain, or both,
512 were retained.

513

514 **Computation of pear orthogroups**

515 To compare the gene content of the two *Pyrus communis* cultivars, 'Bartlett' and 'd'Anjou',
516 orthologous and paralogous gene clusters of Bartlett_v1, Bartlett.DH_v2, and d'Anjou were
517 estimated with OrthoFinder version 1.1.5¹¹² for annotated proteins in all the genomes.

518

519 **Bartlett.DH_v2 genome polishing**

520 To improve the base quality of the publicly available pear reference genome, the *Pyrus*
521 *communis* 'Bartlett.DH_v2' assembly was iteratively polished with two rounds of Pilon (v1.24)⁹⁵
522 using the raw Illumina shotgun reads from the Bartlett.DH_v2 genome projects obtained from
523 the NCBI Short Read Archive (SRA accessions: SRR10030340, SRR10030308), and
524 completeness and accuracy assessed with the BUSCO³⁰ embryophyta_odb10 database.

525

526 **Gene family identification**

527 Coding sequences of candidate genes and their corresponding peptides gleaned from published
528 literature were sorted into pre-computed orthologous gene family clusters of representative 26
529 genomes from land plants using the both BLASTp¹¹³ and HMMER hmmscan¹¹⁴ sequence search
530 option of the *GeneFamilyClassifier* tool implemented in the PlantTribes gene family analysis
531 pipeline (<https://github.com/dePamphilis/PlantTribes>). Classification results, including
532 orthogroup taxa gene counts, corresponding superclusters (super orthogroups) at multiple
533 clustering stringencies, and orthogroup-level annotations from multiple public biological
534 functional databases are reported in Supplementary Table 5.

535

536 **Gene family analysis**

537 All the tools used in this process are modules from the command line version of PlantTribes
538 software and are processed on SCINet (<https://scinet.usda.gov/>) with customized scripts. Protein
539 coding genes from 14 Rosaceae species (*Fragaria vesca* v2.0.a2¹¹⁵, *Rosa chinensis* old Blush
540 homozygous v2.0¹¹⁶, *Rubus occidentalis* v3.0¹¹⁷, *Prunus avium* v1.0.a1¹¹⁸, *Malus domestica*
541 HFTH v1.0¹³, *M. domestica* GDDH13 v1.1¹⁵, *M. domestica* Gala v1.0¹⁴, *M. sieversii* v1.0¹⁴, *M.*
542 *sylvestris* v1.0¹⁴, *Pyrus communis* v1.0¹⁸, *Pyrus communis* Bartlett DH v2.0¹⁷, *Pyrus ussuriensis*
543 *x communis* v1.0¹¹⁹, *Pyrus bretschneideri* v1.1¹²⁰, *Pyrus communis* d'Anjou v0.1) were sorted
544 into orthologous groups with the *GeneFamilyClassifier* as previously described. For species
545 lacking matching coding sequence file and peptide file, transcripts were processed to predict
546 potential protein coding regions using the TransDecoder¹²¹ option of *AssemblyPostProcessor*. A
547 detailed summary of the Rosaceae gene family classification results are in Supplementary Table

548 3. Sequences classified into the orthogroups of interest (with candidate genes in this study) were
549 integrated with scaffold backbone gene models using the *GeneFamilyIntegrator* tool. Gene
550 names were modified as shown in Supplementary Table 9 for easier recognition of the species.
551 Amino acid multiple sequence alignments and their corresponding DNA codon alignments were
552 generated by *GeneFamilyAligner* with the L-INS-i algorithm implemented in MAFFT¹²². Sites
553 present in less than 10% of the aligned DNA sequences were removed with trimAL¹²³.
554 Maximum likelihood (ML) phylogenetic trees were estimated from the trimmed DNA
555 alignments using the RAxML algorithm¹²⁴ option in the *GeneFamilyPhylogenyBuilder*. One
556 hundred bootstrap replicates (unless otherwise indicated) were conducted for each tree to
557 estimate the reliability of the branches. The multiple sequence alignments were visualized in the
558 Geneious R9 software¹²⁵ with Clustal color scheme. The phylogeny was colored with a custom
559 script and visualized with Dendroscope (version 3.7.5)¹²⁶. Gene sequences, alignments, and
560 phylogenies are available in Supplementary File 1-3.

561

562 **Domain prediction**

563 To estimate domain structures of proteins in each orthogroup, the predicted amino acid
564 sequences (either obtained from public databases or generated by the PlantTribes
565 *AssemblyPostProcessor* tool) were submitted to interproscan (version 5.44-79.0)^{127,128} on SCINet
566 and searched against all the databases.

567

568 **Targeted gene family annotation**

569 The following approaches were used in parallel to annotate candidate genes from the original
570 Bartlett.DH_v2, polished Bartlett.DH_v2, and the d'Anjou genome assemblies:

571

572 *TGFam-finder*⁶⁷

573 The ‘RESOURCE.config’ and ‘PROGRAM_PATH.config’ files were generated according to the
574 author’s instruction. The two Bartlett.DH_v2 genome assemblies and the d’Anjou_v1 genome
575 were used as the *target genomes*. Complete protein sequences from apples and pears in the same
576 orthogroup were used as *protein for domain identification*. Complete protein sequences from
577 other Rosaceae species and *Arabidopsis thaliana* in the same orthogroup were used as *resource*
578 *proteins* for each annotation step. For each orthogroup, Pfam annotations from the InterProScan
579 results were used as *TSV for domain identification*. For orthogroups without Pfam descriptions,
580 MobiDBLite information was used as *TSV for domain identification*.

581

582 *Bitacora*⁶⁶

583 Arabidopsis genes from targeted gene families (orthogroups of interest) were used to generate a
584 multiple sequence alignment and HMM profile using MAFFT¹²² and hmmbuild. The resulting
585 files were then used as input for Bitacora v1.3, running in both genome mode and full mode to
586 identify genes of interest in the original Bartlett.DH_v2 genome.

587

588 **Manual curation and gene model verification**

589 In cases where both TGFam-Finder and Bitacora failed to predict a full-length gene, the gene
590 model was curated manually.

591

592 *Curation with orthologous gene models*

593 First, the genomic region containing the target sequence was determined either by the general
594 feature format file (gff) or a BLASTn search using the coding sequence of the target gene or a
595 closely related gene as a query. Next, a genomic fragment containing the target sequence and
596 3kb upstream and downstream of the targeted region was extracted. Then, the incomplete
597 transcript(s), predicted exons, and complete gene models from a closely related species were
598 mapped to the extracted genomic region. The final gene model was determined by using the full-
599 length coding sequence of a closely related gene as a reference.

600

601 *Curation with RNA-seq read mapping*

602 The gff3 files obtained from Bitacora were loaded into an Apollo docker container (v2.6.3)¹²⁹ for
603 verification of the predicted gene models using expression data. Publicly available RNA-seq
604 data⁶⁸⁻⁷³ for *Pyrus* were used as inputs of an RNA-seq aligner, STAR (v2.7.8a)¹³⁰, and
605 alignments were performed with maximum intron size set to 5kb and default settings. Intron-
606 exon structure was compared to the aligned expression data. If there was insufficient RNA-seq
607 coverage from the targeted cultivar, data from other cultivars and *Pyrus* species were used as
608 supporting evidence. Read mapping results are available in Supplementary File 4-5. Curated
609 gene models from the original Bartlett.DH_v2 were transferred to the polished genome for
610 validation.

611

612 Gene model cartoons were generated using the visualize gene structure function in TBtools
613 (v1.09854)¹³¹. Final gene models and their corresponding chromosomal locations are available in
614 Supplementary File 6-7.

615

616 **Data Availability**

617 Raw read data of d'Anjou genome sequencing has been deposited at NCBI SRA under
618 Bioproject ID PRJNA762155. Genome assembly and gene prediction of the draft 'd'Anjou'
619 genome, and the polished 'Bartlett.DH_v2' genome assembly have been submitted to the
620 Genome Database for Rosacea (GDR). Supplementary information accompanies the manuscript
621 on the BioRxiv.

622

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629

630 **Conflict of interests**

631 The authors declare no conflict of interests.

632

633 **Author Contributions**

634 HZ, JW, LH conceived and designed the research. PR prepared gDNA for sequencing. HZ, EW,
635 PT, JE, JW, and AH performed the genome assembly and gene family analysis. All authors
636 participated in writing and revising the manuscript.

637

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928 **Figure legends**

929 **Fig 1. Characterization of the d'Anjou_v1 genome and protein orthology among European**

930 **pears. a.** Dot plot of genome alignment of Bartlett.DH_v2 (x axis) and d'Anjou_v1 (y axis). **b.**

931 Overlap and distinctiveness of gene annotations among three *Pyrus communis* genotypes,

932 Bartlett_v1, Bartlett.DH_v2, and d'Anjou.

933

934 **Fig 2. A workflow for candidate gene identification, curation, and gene family construction.**

935 Grey dotted boxes outlined the three steps of this workflow. Boxes with green outlines are input

936 information. Boxes with blue outlines are intermediate outputs and boxes with purple outlines

937 are final outputs. Contents in boxes with orange outlines are softwares used for generating the

938 outputs.

939

940 **Fig 3. Phylogeny, amino acid sequence comparison, and RNAseq read mapping of *PIN***

941 **genes. a.** One clade of short *PINs* from OG1145 phylogeny. *Malus* genes are indicated with a

942 blue background, *Pyrus* with a green background, and *Prunus* with a pink background. **b.** Amino

943 acid sequence alignment of orthologous genes from 10 Amygdaloideae species in the long *PIN*

944 gene family (OG438). Sites identical to the consensus are shown in grey and sites different from

945 the consensus are shown with a color following the Clustal color scheme in Geneious. Green

946 color in the identity row indicates 100% identical across all sequences and greeny-brown color

947 indicates identity from > 30% to < 100% identity. Gaps in the alignment are shown with a

948 straight line. **c.** RNAseq reads (forward: red; reverse: blue) mapped to a fragment of

949 chromosome 13 in the Bartlett.DH_v2 genome, where a long *PIN* gene,

950 *Pyrco_BartlettDH_13g21160*, was annotated. Gene model in the yellow box is a putative gene

951 model predicted with RNAseq reads (ref. 73) mapped to this region. The two gene models above
952 the read mapping are retrieved from the original annotations of Bartlett_v1
953 (*Pyrco_Bartlett_017869.1*) and Bartlett.DH_v2 (*Pyrco_BartlettDH_13g21160*).

954

955 **Fig 4. Phylogeny, intron-exon structure, and amino acid comparison of IGT genes. a.**

956 Cladogram of the IGT gene family (including LAZY1-like, LAZY5-like, TAC1-like, and DRO-
957 like). Genes are colored as shown in Supplementary Fig. 2. 1000 bootstrap replicates were
958 conducted to estimate reliability and the numbers on the node indicate bootstrap support. **b.**

959 Cartoon illustrating intron-exon structures of IGT genes from *Arabidopsis thaliana* (Araport11),
960 Bartlett.DH_v2, and d'Anjou. **c.** Amino acid alignment of IGT genes from *Arabidopsis thaliana*,
961 Bartlett.DH_v2, and d'Anjou. Sites consisting of a similar amino acid type as the consensus were
962 highlighted with a background color following the Clustal color scheme in Geneious. Red color
963 in the identity row indicates identity < 30%. Five conserved regions were highlighted with a grey
964 symbol below the consensus sequence.

965

966 **Tables**

967 **Table 1. Comparison of genome assembly and annotation, and orthogroups among *Pyrus communis***
 968 **genotypes.**

969

Characteristics	Bartlett_v1	Bartlett.DH_v2	d'Anjou_v1
Assembly			
Assembly size (Mb)	600	507.7	600
Number of scaffolds	142,083	592	5800
Scaffold N50	88 Kb	8.1 Mb	358.88 Kb
Pseudochromosomes	17	17	17
Annotation			
Predicted gene number	43,419	37,445	45,981
BUSCO	96.5%	96.5%	96.6%
Mean CDS length	1209	1120	1343
Gene family classification			
Percentage of genes classified into pear orthogroups	76.2	76.2	80.4
Percentage of pear orthogroups containing genes	93.7	81	90.7
Number of 26Gv2 orthogroups containing genes	9878	9668	9837

970

971 **Table 2. Architecture gene family table**

Family/ Gene	Species	Gene ID	Cultivar/Ecotype	Method	Associated Architecture Phenotype	Refs
PIN						
PIN1	Apple (<i>Malus domestica</i>)	<i>MdPIN1</i> : <i>MDP0000138035</i> (<i>M. domestica</i> genome v1.0)	Royal Gala	Overexpression of <i>Malus PIN1</i> gene in Arabidopsis	Inhibition of primary root elongation, increased lateral root number, enhanced phototropic and geotropism responses.	[51]
		<i>MdPIN1a</i> : <i>MF506847</i> <i>MdPIN1b</i> : <i>MF506848</i> (Genbank)	<u>Rootstocks</u> : Baleng Crab, M9; <u>Interstems</u> : M9; <u>Scion</u> : Red Fuji	Comparative gene expression between cultivars; subcellular localization; overexpression in tobacco; IAA	Lower <i>MdPIN1b</i> expression in bark of M9 dwarfing rootstock and interstem; longer lateral roots, more adventitious roots, shorter and fewer root hairs in <i>MdPIN1b</i> -overexpressing tobacco	[53]

				quantification	lines.	
	Pear (<i>Pyrus communis</i> , <i>P. bretschneideri</i>)	<i>PcPIN-L</i> : PCP021016 (<i>P. communis</i> genome v1.0)	Aihuali x Chili' (<i>P. bretschneideri</i> Rehd.), Aihuali, Chili	Comparative gene expression between cultivars, across tissue types; subcellular localization; promoter activity; overexpression in tobacco, IAA quantification	Lower <i>PcPIN-L</i> expression in leaves, stems, roots, and seeds of pears exhibiting dwarfism; higher IAA content in shoot tips and lower IAA content in stems of pears exhibiting dwarfism; taller plants with longer cells in the stem, longer and more lateral roots in <i>PcPIN-L</i> overexpressing tobacco lines	[58]
PIN1, PIN3	Apple (<i>Malus robusta</i> , <i>M. spectabilis</i>)	<i>MrPIN1</i> : MDP0000138035 <i>MrPIN3</i> : MDP0000497581 (<i>M. domestica</i> genome v1.0)	<u>Rootstock</u> : <i>M. robusta</i> <u>Scion</u> : <i>M. spectabilis</i> Bly114 (Standard) and more-branching mutant (<i>MB</i>)	Gene expression (transcriptome and qPCR) and phenotypes of grafted plants, sugar and hormone quantification across tissues	The more-branching (<i>MB</i>) mutant repressed rootstock growth, and glycolysis and tricarboxylic acid activities. Rootstocks grafted with <i>MB</i> showed reduced <i>MrPIN1</i> expression, and increased <i>MrPIN3</i> expression.	[46]
PIN family	Pear (<i>Pyrus bretschneideri</i> , <i>P. betulifolia</i> , <i>P. communis</i>)	<i>PbPIN1-1</i> : LOC103946937 <i>PbPIN1-2</i> : LOC103950573 <i>PbPIN1-3</i> : LOC103933990 <i>PbPIN1-4</i> : LOC103960490 <i>PbPIN2-1</i> : LOC103941631 <i>PbPIN2-2</i> : LOC103950477 <i>PbPIN3-1</i> : LOC103947028 <i>PbPIN3-2</i> : LOC103948593 <i>PbPIN3-3</i> : LOC103948670 <i>PbPIN4</i> : LOC103931858 <i>PbPIN5-1</i> :	<u>Rootstock</u> : Douli <u>Interstems</u> : OHF51, QN101 <u>Scion</u> : Xueqing	Comparative gene expression between cultivars	Compared <i>PbPIN</i> gene expression among different tissues of dwarfing OHF51 and vigorous QN101 rootstock cultivars, finding differential expression across tissues. Many <i>PbPINs</i> had higher shoot tip expression in the dwarfing rootstocks.	[61]

LOC103930394
PbPIN5-2:
LOC103938552
PbPIN6:
LOC103951142
PbPIN8:
LOC103934837
 (Genbank)

Arabidopsis	<i>AtPIN1: At1g73590</i> <i>AtPIN2: At5g57090</i> <i>AtPIN3: At1g70940</i> <i>AtPIN4: At2g01420</i> <i>AtPIN5: At5g16530</i> <i>AtPIN6: At2g77110</i> <i>AtPIN7: At1g23080</i> <i>AtPIN8: At5g15100</i> (TAIR10)	Review of PIN protein evolution, protein structure, genomic structure, expression patterns, subcellular mechanisms, mutant phenotypes	Mutations in one or more <i>PIN</i> genes can lead to pin-like inflorescences, floral defects, gravitropism defects in the shoot or root, fused leaves or cotyledons, and loss of apical-basal patterning.	[38]
Across kingdom	All plants	Review of the role of auxin transport in branching forms across the plant kingdom	PIN proteins, via polar auxin transport, regulate branch initiation, branch outgrowth, and branch angle in flowering plants.	[48]

AUX/LAX

AUX1, LAX2	Apple (<i>Malus domestica</i>)	<i>MdLAX2:</i> <i>MDP0000020317,</i> <i>MDP0000155074</i> <i>MdAUX1:</i> <i>MDP0000155113</i> (<i>M. domestica</i> genome v1.0)	<u>Rootstock:</u> M9, M27, M793 Scion: Royal Gala	Gene expression (transcriptome and qPCR) comparing dwarfing (M27, M9) and vigorous (M793) rootstocks, carbohydrate analysis	Dwarfing rootstocks exhibited a downregulation of <i>MdAUX1</i> and <i>MdLAX2</i> auxin transporters, among other differentially expressed genes. Further, starch synthesis was upregulated, and dwarfing rootstocks contained higher starch and lower fructose and glucose.	[47]
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AUX1	Apple (<i>Malus domestica</i>)	<i>MdAUX1</i> : <i>MDP0000885425</i> (<i>M. domestica</i> genome v1.0)	Hanfu (diploid and autotetraploid seedlings)	Phenotyping, Gene expression (Tag-seq and qPCR) comparing diploids and autotetraploids	Autotetraploid plants exhibited dwarfism. <i>AUX1</i> was found to be downregulated, in addition to changes in brassinosteroid gene expression (see below).	[45]
AUX1/ LAX family	All plants (focus on models)	<i>AUX1/LAX</i> genes across species		Review	Auxin transport, via <i>AUX1/LAX</i> proteins, PINs, and PGP/ABCs, plays a major role in many architecture-related developmental processes, such as root development (primary and lateral root, gravitropism, root hairs), phyllotactic patterning, leaf morphogenesis, and inflorescence architecture.	[59]
IGT						
TAC1	<i>Prunus persica</i>	<i>PpeTAC1</i> : <i>Ppa010082</i> (<i>P. persica</i> genome v1.0) <i>AtTAC1</i> : <i>At2g46640</i> (TAIR10)	Crimson Rocket	Pnome to map pillar mutation, gene expression, branch and flower bud angle phenotypes, <i>AtTAC1</i> overexpression in Arabidopsis	Pillar peach variety “Crimson Rocket” has insertion within <i>PpeTAC1</i> gene. Overexpression of <i>AtTAC1</i> in Arabidopsis leads to narrow branch angles. <i>PpeTAC1</i> and <i>AtTAC1</i> are expressed in branch tips.	[42]
TAC1, LAZY1, LAZY2	Apple (<i>Malus domestica</i>)	<i>MdoTAC1a</i> : <i>MG837476</i> <i>MdoTAC1b</i> : <i>MG837477</i> <i>MdoLAZY1</i> : <i>MG837478</i> <i>MdoLAZY2</i> : <i>MG837479</i> (Genbank)	<u>Rootstock</u> : <i>Malus robusta</i> <u>Scion</u> : McIntosh, Wjczik, Granny Smith, Fukushima spur	Gene expression (qPCR) comparing cultivars representative of four architectural ideotypes.	Comparison of <i>IGT</i> gene expression between McIntosh (standard), Fukushima spur (spur), Granny Smith (tip- bearing), and Wjczik (columnar), showed decreasing levels of <i>TAC1</i> in shoot tips in that cultivar order, with the lowest expression of all identified <i>IGT</i> genes in the columnar cultivar in all tissues.	[55]

DRO1	Apple (<i>Malus domestica</i>)	<i>MdDRO1</i> : MDP0000142588 <i>MdDRO2</i> : MDP0000151294 <i>MdDRO3</i> : MDP0000723826 <i>MdPIN11</i> : MDP0000125862 (<i>M. domestica</i> genome v1.0)	<u>Rootstock</u> : M9, Baleng Crab and interspecific hybrids from M9 x BC cross (rooted cuttings)	Root angle phenotyping, gene expression (qPCR in roots tissue), IAA quantification	Deep-rooted Baleng Crab had steeper root angles, and greater root length than M9. M9 showed lower IAA content, higher IAA oxidase activity, fewer amyloplasts, and lower expression of <i>MdDRO1</i> and <i>MdPIN11</i> architecture- associated genes.	[50]
	Plum (<i>Prunus domestica</i>), Peach (<i>Prunus persica</i>), Arabidopsis	<i>PpeDRO1</i> : Ppa021925 (<i>P. persica</i> genome v1.0) <i>AtDRO1</i> : At1g72490 <i>AtDRO2</i> : At1g19115 <i>AtDRO3</i> : At1g17400 (TAIR10)	Plum seedlings: Stanley Arabidopsis: Columbia	Gene expression (qPCR, GUS), root angle phenotyping, root gravitropism, <i>DRO1</i> overexpression in plum and Arabidopsis	<i>DRO1</i> and <i>DRO2</i> are expressed in roots in Arabidopsis and peach. atdro1 mutants had wider lateral root angles, but no difference in primary root gravitropism. <i>AtDRO1</i> OE Arabidopsis had narrower lateral root branch angles, distinct upward leaf curling, shorter siliques, narrower shoot branch angle. <i>PpeDRO1</i> OE plum roots were longer with greater root/shoot weight than controls.	[49]
IGT Family	Across kingdom	<i>IGT</i> gene across species		Review of <i>IGT</i> gene studies from the 1930's to the present, phylogenetic analysis	<i>IGT</i> genes influence growth angle/gravitropic set point angle of both shoots and roots, which affects access to water and nutrients, density of plantings, structural integrity and soil anchorage, and overall crop productivity.	[34]

GID1

GID1c	Peach (<i>Prunus persica</i>)	<i>PpeGID1c</i> : <i>Ppa018174</i> (<i>P. persica</i> genome v1.0)	Brachytic dwarf (BD), Standard, and a mapping population from a BD x Std cross	Pnome to map <i>dw</i> mutation, phenotyping and GA application response, RNAi silencing of <i>GID1c</i> in plum, gene expression (qPCR)	BD peaches exhibit extreme dwarfism, primarily attributed to reduced internode length. The <i>dw</i> mutation in BD peaches was mapped to <i>PpeGID1c</i> . BD peaches showed insensitivity to GA treatment. Silencing <i>GID1c</i> in plums led to a BD-like dwarfed phenotype.	[44]
			FenHuaShouXing Tao (FHSXT), QiuMiHong (QMH)	Phenotyping of dwarf (FHSXT) and Standard (QMH) cultivars, GA response and quantification, gene expression (qPCR), yeast-2-hybrid, protein quantification (western)	FHSXT exhibited extreme dwarfism, short internodes, shorter cell length, fewer branches, and longer leaves. FHSXT had high levels of GA and were GA insensitive. <i>GID1c</i> was upregulated in FHSXT, as were multiple GA biosynthesis genes (see below). The mutation in FHSXT <i>GID1c</i> abolished interaction with DELLA1 in a yeast-2-hybrid assay.	[56]
GID1	Arabidopsis	<i>AtGID1a</i> : <i>At3g05120</i> , <i>AtGID1b</i> : <i>At3g63010</i> , <i>AtGID1c</i> : <i>At5g27320</i> (TAIR10)	Columbia	Phenotyping mutants, gene expression (qPCR), GA treatment and quantification	Double mutants <i>atgid1a atgid1c</i> and <i>atgid1a atgid1b</i> showed reduced stem height phenotypes, while the triple mutant exhibited severe dwarfing and GA insensitivity. The triple mutant phenotype was partially rescued by loss of function of the DELLA protein AtRGA.	[36]
GA2ox, GA3ox, GA20ox						
GA2ox	Peach (<i>Prunus persica</i>)	<i>PpeGA2ox-1</i> : <i>Prupe.1G111900</i> <i>PpeGA2ox-2</i> : <i>Prupe.1G344000</i> <i>PpeGA2ox-3</i> : <i>Prupe.3G006700</i> <i>PpeGA2ox-4</i> : <i>Prupe.4G026300</i>	QiuMiHong (QMH)	Gene expression (qPCR), <i>PpeGA2ox</i> gene overexpression in tobacco, GA treatment and quantification	Seven <i>GA2ox</i> genes were identified in peach and classified into three subgroups, and tissue-specific expression was determined in a standard peach cultivar. Overexpression of	[63]

		<p><i>PpeGA2ox-5:</i> <i>Prupe.4G080700</i> <i>PpeGA2ox-6:</i> <i>Prupe.4G150200</i> <i>PpeGA2ox-7:</i> <i>Prupe.4G204600</i> (<i>P. persica</i> genome v2.0)</p>			<p><i>PpeGA2ox1</i>, <i>PpeGA2ox5</i>, and <i>PpeGA2ox2</i> resulted in dwarf phenotypes in tobacco. GA treatment at shoot tips induced expressions of all <i>PpeGA2ox</i> genes, but at different rates.</p>	
	<i>Arabidopsis thaliana</i>	<p><i>AtGA2ox1:</i> <i>At1g78440</i> <i>AtGA2ox2:</i> <i>At1g30040</i> <i>AtGA2ox3:</i> <i>At2g34555</i> <i>AtGA2ox4:</i> <i>At1g47990</i> <i>AtGA2ox6:</i> <i>At1g02400</i> <i>AtGA2ox7:</i> <i>At1g50960</i> <i>AtGA2ox8:</i> <i>At4g21200</i> (TAIR10)</p>	Columbia-o and <i>hy5</i> mutant	Gene expression (GUS reporters, qPCR), <i>GA2ox</i> overexpression	Overexpression of <i>GA2ox</i> genes inhibited elongation of the hypocotyl, rescued the long-hypocotyl phenotype of <i>hy5</i> mutants. Overexpression of <i>GA2ox7</i> and 8 led to extra shortening of the hypocotyl in <i>hy5</i> mutants.	[57]
GA20ox	Apple (<i>Malus domestica</i>)	<p><i>MpGA20ox1A</i> <i>MpGA20ox1B</i> (Noted from <i>M. pumila</i>, a previous name for <i>M. domestica</i>. Gene IDs unclear)</p>	<p><u>Rootstock:</u> M25, MM106 <u>Scion:</u> Greensleeves</p>	Gene expression (qPCR), gene silencing, phenotyping, grafting onto invigorating rootstocks, GA quantification	Silencing of <i>MpGA20ox1A</i> and <i>B</i> led to reduced height, and reduced internode length and number. Application of exogenous GA rescued the dwarfed phenotype. Transgenic dwarfed scions remained dwarfed after grafting onto invigorating rootstocks.	[35]
GA2ox, GA3ox, GA20ox	Peach (<i>Prunus persica</i>)		FenHuaShouXing Tao (FHSXT), QiuMiHong (QMH)	See above (<i>GID1</i>)	Expression of several <i>GA2ox</i> , <i>GA3ox</i> , and <i>GA20ox</i> GA biosynthesis genes were upregulated in a dwarfed variety (FHSXT), as was <i>GID1c</i> (see above).	[56]

WRKY9

WRKY9	Apple (<i>Malus domestica</i>)	<i>MdWRKY9</i> : <i>MDP0000272940</i> (<i>M. domestica</i> genome v1.0)	<u>Dwarfing rootstock</u> : M.9 Pajam 2, M26, GM256, B9 and M.9 T337 <u>Non-dwarfing rootstock</u> : <i>M. baccata</i> , <i>M. robusta</i> , <i>M. sieversii</i> , <i>M. prunifolia</i> , <i>M. zumi</i>	Gene expression (qPCR), <i>MdWRKY9</i> overexpression in apple, subcellular localization, transcriptional inhibition assays, hormone quantification, CHIP-qPCR	<i>MdWRKY9</i> was more highly expressed in dwarfing rootstock. Overexpression of <i>MdWRKY9</i> in M26 semi-dwarfing rootstock resulted in dwarfed characteristics, and fewer, but longer roots. <i>MdWRKY9</i> binds and inhibits expression of the brassinosteroid biosynthetic gene <i>MdDWF4</i> (see below)	[54]
EIN2						
EIN2	Rice (<i>Oryza sativa</i>)	<i>OsEIN2</i> : <i>LOC_Os07g06130</i> <i>OsEIL1</i> : <i>LOC_Os03g20790</i> (MSU Rice Gene Models)	Nipponbare	Computed tomography (CT), mutant analysis, confocal microscopy, ethylene treatment and quantification, gas barrier treatment, GUS assay, ethylene biosensor	Ethylene-insensitive signaling mutants (<i>osein2</i> and <i>oseil1</i>) were unaffected by and grew deeper into compacted soil. Shoot and root biomass was increased in ethylene-insensitive mutants grown in compacted soil. Ethylene was slow to diffuse through compacted soils and ethylene detection in the root was shown to increase in compacted soil.	[64]
	<i>Arabidopsis thaliana</i>	<i>AtEIN2</i> : <i>At5g03280</i> (TAIR10)	Columbia	Gene expression (GUS), root architecture phenotyping on differing Boron concentrations, mutant analysis	Ethylene-insensitive mutant <i>atein2-1</i> inhibited increased root hair formation and elongation under low-Boron conditions.	[39]

	All species	Ethylene signaling and biosynthesis genes		Review of ethylene effects on root and hypocotyl elongation, root hair formation, apical hook formation, stem growth, biosynthesis pathway, and signaling pathway	Inhibitory effects of ethylene on root growth observed as early as 1901. Plant grown on aerated soils have high ethylene and strong root growth inhibition when treated with ethylene. Ethylene interacts with GA and auxin pathways to regulate root growth, as well as JA and auxin pathways to regulate root hair formation. Ethylene plays a role in inhibiting stem growth, and can also stimulate stem growth in shaded conditions.	[37]
DWF1, DWF4						
DWF1, DWF4	Apple (<i>Malus domestica</i>)	<i>MdDWF1-1</i> : MD13G1007700 <i>MdDWF1-2</i> : MD16G1000400 <i>MdDWF4-1</i> : MD02G1149000 <i>MdDWF4-2</i> : MD15G1263900 <i>MdDWF4-3</i> : MD17G1120200 (<i>M. domestica</i> GDDH13 genome v1.1)	Rooted seedlings: M.9-T337, Yanfu No. 6 (YF), Nagafu No. 2 (CF), <i>M. hupehensis</i> Rootstock: M.9, CF Scion: CF	Phylogeny, synteny analysis, gene expression (qPCR), hormone treatment and quantification	YF trees are spur type and have lower shoot elongation rate, number of internodes, and average internode length relative to CF. <i>mddwf1-1</i> and <i>mddwf1-2</i> are highly expressed in CF relative to YF. CF/M.9 (dwarf) trees have lower <i>mddwf4</i> and <i>mddwf1</i> expression relative to CF/CF.	[52]
DWF4	Apple (<i>Malus domestica</i>)	<i>MdDWF4</i> : MDP0000498540 (<i>M. domestica</i> genome v1.0)	<u>Dwarfing rootstock</u> : M.9 Pajam 2, M26, GM256, B9 and M.9 T337 <u>Non-dwarfing rootstock</u> : <i>M. baccata</i> , <i>M. robusta</i> , <i>M. sieversii</i> , <i>M. prunifolia</i> , and <i>M. zumi</i>	Gene expression (qPCR), <i>MdWRKY9</i> overexpression in apple, subcellular localization, transcriptional inhibition assays, hormone quantification, CHIP-qPCR	Overexpression of <i>MdWRKY9</i> in apple resulted in significantly lower expression of <i>mddwf4</i> and lower brassinosteroid content. <i>MdWRKY9</i> binds and inhibits expression of the brassinosteroid biosynthetic gene <i>MdDWF4</i> (see above).	[54]

DWF1, DWF4	Arabidopsis, Tomato	<i>AtDWF1</i> : <i>At3g19820</i> <i>AtDWF4</i> : <i>At3g50660</i> (TAIR10)		Review of multiple BR biosynthetic genes, pathways, and mutant phenotypes and chemicals that led to these findings	<i>dwf</i> mutants exhibit extreme dwarfism, with additional alteration in leaf and inflorescence development. DWARF1 is a biosynthetic enzyme involved in reducing C24 of BR. DWARF4 is a cytochrome P450 enzyme involved in hydroxylating C22 of BR and represents a rate-limiting step in biosynthesis.	[33]
IPT3						
IPT3	Apple (<i>Malus domestica</i>)	<i>MdPIN1</i> : Unclear. Degenerate primers designed based on Arabidopsis, lupin, pea, and <i>Populus</i> sequences. <i>MdIPT3</i> : Unclear. Degenerate primers designed based on <i>Malus hupehensis</i> , Arabidopsis, and cabbage sequences.	<u>Rootstock</u> : M9 and MM (<i>M. x micromalus</i>) <u>Scion</u> : Red Fuji <u>Interstock</u> : M9	Gene expression (qPCR), hormone quantification, grafting substitution experiments (rootstock substitutions, rootstock bridging, and bark substitutions)	M9 (common dwarfing rootstock) seedlings showed lower <i>PIN1</i> and <i>IPT3</i> expression, as well as lower auxin and zeatin across different plant tissues, compared with MM seedlings. Grafting combinations including M9 as rootstock or standard interstock similarly frequently had reduced <i>PIN1</i> and <i>IPT3</i> expression and reduced hormone levels compared with MM grafting combinations. The reduced levels were largely rescued when MM or Red Fuji were introduced as rootstock substitutions, bridged, or bark substitutions.	[40]
	Arabidopsis	<i>AtIPT3</i> : <i>At3g63110</i> <i>AtIPT5</i> : <i>At5g19040</i> <i>AtAHK2</i> : <i>At5g35750</i> <i>AtAHK3</i> : <i>At1g27320</i> <i>AtAHK4</i> : <i>At2g01830</i> (TAIR10)	Col-0	Root architecture phenotyping, gene expression (GUS), hormone treatments, overexpression of cytokinin catabolism genes, mutant	Increasing concentrations of cytokinin led to decreases in lateral root (LR) density. Loss of <i>IPT3</i> and other <i>IPT</i> (cytokinin biosynthesis) genes resulted in increased LR formation and density. Similarly, loss of <i>AHK</i>	[41]

analysis of
biosynthesis
genes cytokinin receptor genes
led to increased LR
densities.

MAX1, MAX2, MAX3, MAX4

MAX1, MAX2, MAX3, MAX4	Apple (<i>Malus domestica</i>)	<i>MdMAX1-1</i> : MDP0000130133 <i>MdMAX1-2</i> : MDP0000677258 <i>MdMAX1-3</i> : MDP0000909874 <i>MdMAX2-1</i> : MDP0000466825 <i>MdMAX3-1</i> : MDP0000197409 <i>MdMAX3-2</i> : MDP0000139334 <i>MdMAX4-1</i> : MDP0000227870 <i>MdMAX4-4</i> : Unclear (Based on primer sequences, <i>M. domestica</i> genome v1.0)	<u>Standards</u> : McIntosh genotypes 21-S, 31-S, and 77-S <u>Columnar apples</u> : Wijcik genotypes 21-C, 31-C, 77-C	Gene expression (qPCR) comparing standard and columnar phenotypes, strigolactone quantification, overexpression of <i>MdCo31</i> in tobacco	<i>MdMAX</i> gene expression was frequently higher in columnar apple buds and shoots, as was expression of <i>MdCo31</i> . Overexpression of <i>MdCo31</i> in tobacco resulted in plants with reduced height, internode lengths, and increase leaf thickness and chlorophyll content. Tobacco <i>MAX3</i> was increased in these lines, while tobacco <i>MAX1</i> was upregulated only in the line with the shortest stature.	[62]
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Peach (<i>Prunus persica</i>)	<i>PpeMAX1</i> : Ppa003950m <i>PpeMAX2</i> : Ppa002017m <i>PpeMAX3</i> : Ppa017865m <i>PpeMAX4</i> : Ppa006042m (<i>P. persica</i> genome v1.0)	<u>Rootstock</u> : Lovell, Bailey, Tennessee natural <u>Scion</u> : Redhaven (standard), Harrow Beauty (standard), Bounty (standard), Crimson Rocket (pillar), Sweet-N- Up (upright)	Gene expression (qPCR), hormone quantification between standard, upright, and pillar growth habits	<i>PpeMAX3</i> and <i>PpeMAX4</i> expression was higher in in stems following pruning. <i>PpeMAX3</i> expression and auxin concentrations were greater in the roots of pillar phenotype. <i>PpeMAX1-4</i> expression was intermediate between pillar and standard. Expression of <i>PpeMAX1</i> , <i>PpeMAX2</i> , and <i>PpeMAX4</i> in roots were higher in pillar, but not statistically significant.	[43]
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MAX1, MAX2	Apple (<i>Malus spectabilis</i> , <i>M. domestica</i> , and <i>M. robusta</i>)	<i>MsMAX2</i> : Used primers based on <i>MdMAX2</i> (<i>MD17G1266700</i>) <i>MsMAX1</i> : Used primers based on <i>MdMAX1</i> (<i>MD15G1057600</i>) (<i>M. domestica</i> GDDH13 genome v1.1)	<u>Rootstock</u> : <i>M. robusta</i> <u>Scion</u> : More-Branching (<i>MB</i>) mutant and wild type (WT) of <i>M. spectabilis</i> cultivar Bly114, <i>M. domestica</i> cultivars T337, M26, and Fuji Nagafu2	Morphological and anatomical phenotypes, hormone quantification, gene expression (transcriptome and qPCR)	<i>MB</i> mutants had decreased height, increased branch number, and narrower branch angles. <i>MsMAX1</i> and <i>MsMAX2</i> were upregulated in axillary buds during outgrowth.	[60]
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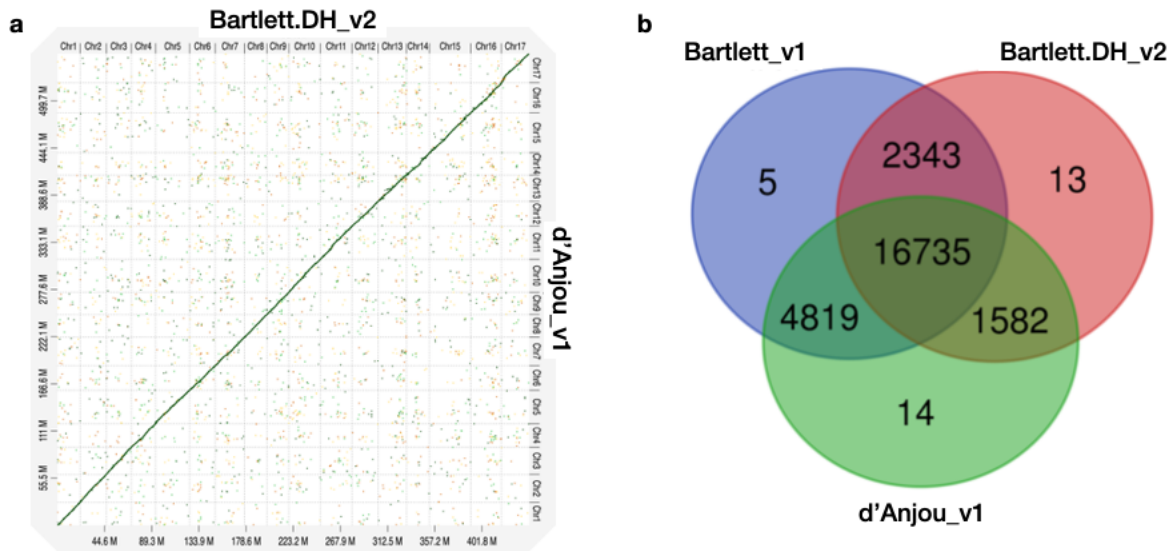
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974 **Figures**

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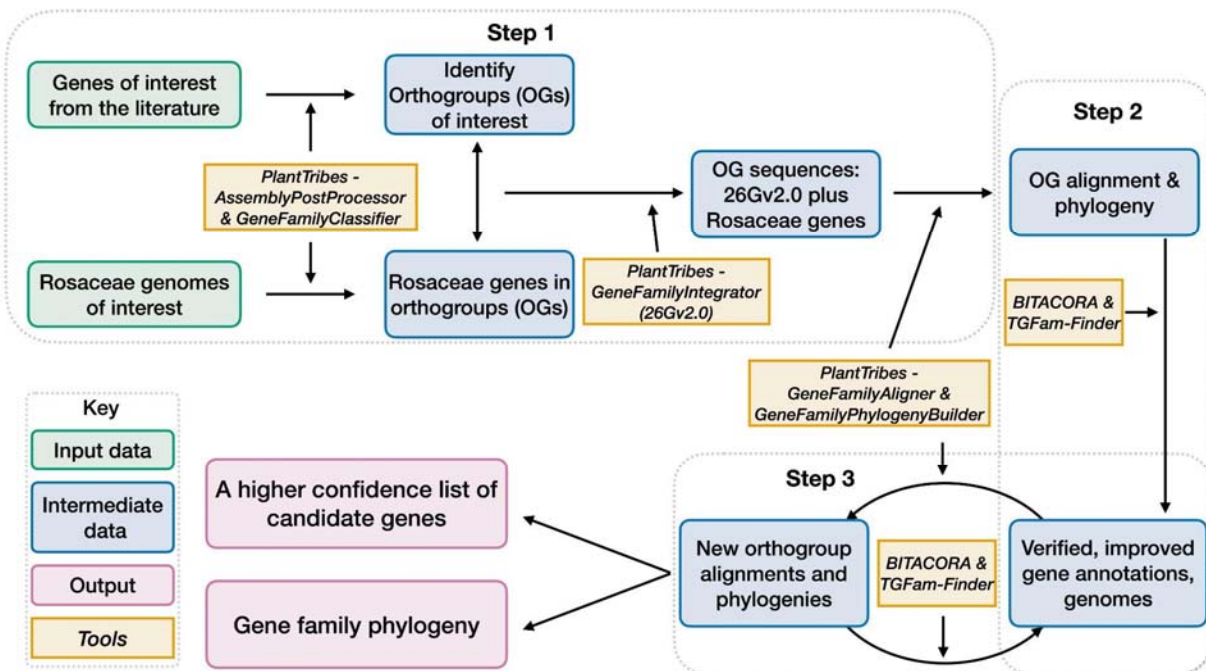
976 Figure 1



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979 Figure 2



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986 Figure 4

