

# 1 **SI Appendix**

## 2 **Draft genome of the peanut A-genome progenitor (*Arachis*** 3 ***duranensis*) provides insights into geocarpy, oil biosynthesis and** 4 **allergens**

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34 *SI Text*

## 35 **1. Sequencing and assembly of *Arachis duranensis***

### 36 **1.1 Plant material**

37 *Arachis duranensis* (AA 2n=2x=20) is the progenitor species of the cultivated  
38 peanut<sup>1,2</sup> (**Fig. S1**). The *A. duranensis* (represented as accession PI475845) was  
39 sequenced by Illumina HiSeq2500 sequencing platform. Genomic DNA was extracted  
40 from the etiolated leaves of 20-day-old plants growing in dark chamber using the  
41 CTAB method<sup>3</sup>.

### 42 **1.2 Illumina shotgun sequencing**

43 Genomic DNA was isolated from caulicle, leaf and root by standard molecular  
44 biology techniques. Subsequently, short-insert libraries (250-bp, 500-bp & 800-bp)  
45 and long-insert libraries (2-kb, 5-kb, 10-kb & 20-kb for BP) were constructed using  
46 the standard protocol provided by Illumina (San Diego, USA). Paired-end sequencing  
47 with whole genome shotgun sequencing strategy was performed using the Illumina  
48 HiSeq 2500 platform. We finally obtained ca. 229.94G reads for next filter step  
49 (**Table S1**).

### 50 **1.3 *De novo* assembly of the *A. duranensis* genome**

51 The schematic strategy for *de novo* assembly is displayed in **Fig. S2**. Sequencing  
52 errors will largely disturb the short-read assembly algorithms. We therefore utilized  
53 several highly stringent filtering steps to remove low-quality reads as follows: (1)  
54 reads of short-insert libraries were trimmed of 4 low-quality bases at both ends, and  
55 reads of long-insert libraries were trimmed of 3 low-quality bases; (2) for long-insert  
56 libraries, duplicated reads were filtered out; (3) we also examined individual reads in  
57 all lanes, and discarded reads with 10 or more Ns (no sequenced bases) and low-  
58 quality bases.

59 We finally obtained 159.07G filtered reads for genome assembling. We employed  
60 SOAPdenovo2<sup>4</sup> (version 2.04.4) with optimized parameters (pregraph -K 79 -p 16 -d  
61 5; scaff -F -b 1.5) to construct contigs and original scaffolds. This paired-end  
62 information was subsequently applied to link contigs into scaffolds in a stepwise  
63 manner. Several intra-scaffold gaps were filled by local assembly using the reads in a  
64 read-pair where one end uniquely mapped to a contig whereas the other end was  
65 located within a gap. Subsequently, SSPACE<sup>5</sup> (version 2.0; using core parameters “-k  
66 6 -T 4 -g 2”) was used to link the SOAPdenovo2 scaffolds. Overall, various assembly  
67 software were employed to generate a draft genome of *A. duranensis* consisting of  
68 8,173 scaffolds with a total of 1,051,523,805 bp (avg. size: 128,658; N50 size:  
69 649,840) and 90,568 contigs (N50 size: 29,584) (**Table 1** and **Table S2**). Out of 8,173  
70 scaffolds, 3,996 with length  $\geq 2$  Kb account for 1.048 Gb of the genome (**Table S3**).

71

## 72 **1.4 Evaluation of the assembly**

### 73 **1.4.1 PCR amplification**

74 We evaluated the *A. duranensis* assembled genome using PCR method. A total of 411  
75 genomic fragments from the assembled genome were randomly selected for designing  
76 PCR primers. Of the 411 pairs of primers, ~89% can be amplified the right size of  
77 product from the genomic DNA of PI475845 (**Table S4** and **Fig. S3**). All primers used  
78 in this study were provided in **Dataset S1**.

### 79 **1.4.2 Per-base accuracy of read data and sequence depth**

80 The accuracy of a genome assembly depends partially on the high quality of  
81 sequenced reads, which has a great impact on subsequent analyses. Base errors in the  
82 sequenced fragments can not only lead to the deviation of assembly, but also result in  
83 the incorrect annotation of functional elements in downstream analyses. The read  
84 length and quality distributions were thus explored (**Fig. S4**). Nearly all reads below

85 1000 bp have high quality ( $Q > 20$ ). The high-quality data guarantees the single base  
86 accuracy of the assembled genome and the correct annotation of functional elements  
87 like protein-coding genes, transcription factors and small RNAs. The sequencing depth  
88 of 93.27% genomic regions was  $\geq 10x$  and its peak locates at 48x (**Fig. S5**), indicating  
89 that these regions had high single-base accuracy<sup>6</sup>.

#### 90 **1.4.3 EST and Transcriptome Sequence Assembly (TSA) mapping**

91 The gene coverage of the assembled genome was comprehensively evaluated using  
92 available transcript sequence tags or ESTs. We used the RNA-seq data<sup>7</sup> generated in-  
93 house and downloaded from the Sequence Read Archive (SRA)  
94 (<http://www.ncbi.nlm.nih.gov/Traces/sra/>). We aligned the transcripts to the genome  
95 using SSAHA2<sup>8</sup> with default parameters except for '-best 1'. A total of 50,281  
96 (approximately 99% of the predicted genes) genes were supported by at least one  
97 transcripts (**Fig. S6**).

#### 98 **1.5 Estimation of the genome size based on 25-mer analysis**

99 The genome size was estimated based on the K-mer distribution using ~79 Gb of  
100 high-quality short reads. A k-mer refers to a total number of sub-sequences of length k  
101 which could be obtained from a sequenced DNA read. The genome size was evaluated  
102 using the total length of sequence reads divided by sequencing depth. To estimate the  
103 sequencing depth, the frequency of each 25-mer were calculated from the whole  
104 genome sequenced reads. We used the algorithm:  $(N \times (L - K + 1) - B) / D = G$ , where  
105 N is the total sequence read number, L is the average length of sequence reads and K  
106 is K-mer length, defined as 25 bp here, B is the total number of low frequency 25-  
107 mer, G denotes the genome size, and D is the overall depth estimated from K-mer  
108 distribution (**Table S7**). An average of 57.14x read depth was obtained with an  
109 estimated genome size of 1,381,794,909 bp, consistent with the prior data<sup>9</sup>.

110

## 111 **2. Genome annotation**

### 112 **2.1 Gene prediction**

113 To annotate the *A. duranensis* genome, we used an automated genome annotation  
114 pipeline MAKER<sup>10</sup> which aligns and filters EST and protein homology evidence,  
115 produces *de novo* gene prediction, infers 5' and 3' UTR, and integrates these data to  
116 generate final downstream gene models with quality control statistics. Several  
117 iterative runs of MAKER were used to produce the final gene set. In total, 50,324  
118 gene models for *A. duranensis* were predicted in this study (**Table 1**).

### 119 **2.2 Gene function annotation**

120 All predicted protein sequences were functionally annotated using the BLAST+  
121 (version 2.2.27) with a threshold E-value of 1e-5 against a variety of protein and  
122 nucleotide databases, including the NCBI nucleotide (NT), the non-redundant protein  
123 (NR), the Conserved Domain Database (CDD)<sup>11</sup>, the UniProtKB ([www.uniprot.org](http://www.uniprot.org)),  
124 Pfam<sup>12,13</sup> and the Gene Ontology (GO)<sup>14</sup>. The *A. duranensis* genes were also mapped  
125 to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway maps of KEGG  
126 databases<sup>15</sup>. To infer functions for the predicted genes, InterProScan<sup>16,17</sup> was used to  
127 search the predicted genes against the protein signature from InterPro with default  
128 parameters. Fifteen gene sets from legumes, oilseed crops and other plant species  
129 were used for comparative analysis (**Table S8**). A Cytoscape plugin BiNGO was used  
130 for enrichment analysis with hypergeometric test and Benjamini multiple testing  
131 correction at a significance level of 0.01<sup>18</sup>.

### 132 **2.3 Identification of gene and transcription factor families**

133 Comparative analysis of gene family evolution including expansion, contraction,  
134 formation or extinction can reveal evolutionary events underlying species  
135 adaptation<sup>19</sup>. The software OrthoMCL (version 2.09)<sup>20</sup> was employed to identify  
136 orthologous gene families in the *A. duranensis* genome. To cluster protein-coding

137 genes into gene families, pairwise sequence similarity analysis was performed using  
138 BLASTP with an E-value cutoff of 1e-5 and a minimum aligned coverage of 50%.  
139 The reciprocal best hit matrix served as the basis for ortholog definition using  
140 OrthoMCL. The gene sets used in this study are listed in **Table S8**. A total of 832,953  
141 sequences from sixteen plant species were grouped into 54,384 gene clusters, of  
142 which 4,575 clusters contained 237,686 genes common to all sixteen genomes, and  
143 1,423 were specific for *A. duranensis*, suggesting that new gene families may have  
144 emerged after *Arachis* divergence from other legumes ~50 Mya<sup>21</sup>. These specific  
145 clusters are comprised of 16,472 genes, more than in other examined species except  
146 canola (**Table S13** and **Fig. S19**). Gene Ontology (GO) annotation indicated  
147 differentially enriched functional categories in peanut-specific families (**Fig. S20** and  
148 **S21**), suggesting that new gene families may reflect *Arachis* speciation and adaptation  
149 to specific habitats, for example by geocarpy. Legumes shared 6,508 (114,289 genes)  
150 families (**Fig. S15**), while 8,347 (130,529 genes) and 7,117 (113,667 genes) families  
151 were shared with oilseeds and other distantly related species, respectively (**Fig. S16**  
152 and **S17**). Shared and unique gene families are shown in **Figs. S15-S17**.

153 The gene numbers of orthologous families were used to determine the family size  
154 by counting the incorporated *A. duranensis* genes for each cluster. We compared the  
155 *A. duranensis* gene family size relative to corresponding gene family size in other  
156 plant species examined. The number difference of the gene family size and gene copy  
157 number were calculated. Then, the median of the *A. duranensis* gene count was  
158 determined and a polynomial fit of these values was computed using locally-weighted  
159 polynomial regress using an R stats package ([http://stat.ethz.ch/R-manual/R-](http://stat.ethz.ch/R-manual/R-patched/library)  
160 [patched/library](http://stat.ethz.ch/R-manual/R-patched/library)). A comparison of *A. duranensis* gene family size relative to  
161 corresponding gene family size in soybean and *Medicago* was presented in **Table S14**,  
162 indicating that approximately 56% of families showed no change in size between *A.*  
163 *duranensis* and soybean, while 73% between *A. duranensis* and *Medicago*, suggesting

164 that expansion and contraction of *A. duranensis* gene families are different from other  
165 legumes.

166 Transcription factors (TFs) can regulate the expression of genes at the  
167 transcriptional level. For the identification of known TFs in *A. duranensis*, TFs from  
168 other species were retrieved from PlantTFDB (<http://planttfdb.cbi.pku.edu.cn/>)  
169 (**Dataset S5**). For *A. duranensis*, we utilized the predicted gene set against the  
170 PlantTFDB databases using BLASTP with an E-value cutoff of 1e-5. A total of 5,251  
171 TFs were identified in *A. duranensis*, consisting of 58 families, representing 10.43%  
172 of predicted protein-coding genes (**Dataset S5**). Particularly enriched are TF families  
173 such as B3, bHLH, C2H2, C3H, ERF, G2-like, HD-Zip, M-type, YB-related, TCP,  
174 Trihelix and WRKY.

#### 175 **2.4 Identification of non-coding RNAs**

176 Non-coding RNAs include highly abundant and functionally important RNAs. In this  
177 study non-coding RNA genes refer to four different types: transfer RNA (tRNA),  
178 ribosomal RNA (rRNA), microRNA (miRNA) as well as small nuclear RNAs  
179 (snRNAs). Non-coding RNAs were annotated by aligned our assembly to against the  
180 Rfam database (version 11.0)<sup>22</sup>. Three RNA prediction programs including tRNAscan-  
181 SE, RNAmmer and INFERNAL were used to predict the non-coding RNAs in *A.*  
182 *duranensis*. The tRNAs were predicted using the tRNAscan-SE<sup>23</sup>, rRNAs were  
183 identified using the RNAmmer<sup>24</sup>, snRNAs were annotated using the INFERNAL  
184 (version 1.0)<sup>25</sup> and other non-coding RNA genes were annotated by aligning the  
185 genome sequences against Rfam database (version 11.0). Conserved miRNAs were  
186 identified by mapping all entries in miRBase against the assembled genome. Novel  
187 miRNAs were identified using miREAP<sup>26</sup>.

188 In *A. duranensis*, we predicted a total of 913 tRNAs with an average length of  
189 ~73 bp; 115 rRNAs with an average length of ~1 kb, including 5S (61), 5.8S (17), 18S  
190 (21) and 28S (16) as well as 202 snRNAs with an average length of ~127 bp (**Table**

191 **S15).** A total of 816 miRNAs, including 801 conserved belonging to 96 families  
192 (**Tables S15-S16; Dataset S6**) i.e., more than soybean (390 genes, 85 families)  
193 *Medicago* (512 genes, 101 families) (miRBase release 21).

## 194 **2.5 Annotation of repetitive sequences and transposon elements**

195 We examined the genomic positions of the repeats that were classified as Long  
196 Terminal Repeats (LTR), Long Interspersed Nuclear Elements (LINE), Short  
197 Interspersed Nuclear Elements (SINE) and DNA transposons. Repetitive sequences in  
198 *A. duranensis* were identified using the RepeatMasker, Tandem Repeats Finder  
199 (TRF)<sup>27</sup> and RepeatModeler open-1.0<sup>28</sup> for homolog and *de novo* prediction,  
200 respectively. We screened the genome using RepeatMasker against the RepBase  
201 (version 20110920)<sup>29</sup>. The TE sequences were classified according to the unified  
202 classification system<sup>30</sup>. Gaps in the sequences were not included when calculating the  
203 total TE contents. A total of 20,597 scaffolds were subjected to the TE identification,  
204 90.2% (18,580) of which were identified as TE sequences. The remained scaffolds  
205 without TE could be low-copy sequences or contained uncharacterized repeat  
206 sequences so far. Approximately 60% of the *A. duranensis* genome were identified to  
207 be TE sequences (**Fig. 1** and **Table 2**).

## 208 **2.6 Dating the insertion time of LTR retrotransposons**

209 LTR retrotransposons are the most common type of TEs in plants and play a vital  
210 evolutionary role in the remarkable divergence of genome size in flowering plants<sup>31</sup>.  
211 The identity of both ends of LTR can be used to estimate their insertion time in  
212 genome<sup>32</sup>. We used CD-HIT program<sup>33</sup> to cluster LTR retrotransposons based on 90 %  
213 sequence similarity (-c 0.9). The longest sequence of each cluster was chosen as the  
214 representative sequence, and other sequences within the same cluster must cover 90%  
215 of the length of the representative sequence (-aL 0.9). Insertion dates were calculated  
216 using the Kimura two-parameter method<sup>34</sup> with the mutation rate of  $1.3 \times 10^{-8}$



217 substitutions per site per year<sup>35</sup>. The insertion times of LTR retrotransposons were  
218 dated to observe the activity of these elements in the *A. duranensis* genome expansion  
219 regarding the genome structural variations. The histograms, presented in **Fig. S24**,  
220 showed one peak of the insertion times of LTR retrotransposons, revealing these LTR  
221 retrotransposons have undergone one burst of amplification ~2 Mya, suggesting that  
222 the expansion of the *A. duranensis* genome was relatively recent.

223

### 224 **3. Molecular marker development**

#### 225 **3.1 Simple sequence repeats (SSRs)**

226 Simple Sequence Repeats (SSRs) in *A. duranensis* were identified using MISA, a  
227 MicroSATellite identification tool<sup>36</sup> (<http://pgrc.ipk-gatersleben.de/misa/>). SSRs with  
228 di-nucleotide motifs were defined with at least 6 repeats and 5 repeats for tri-, tetra-,  
229 penta- and hexa-nucleotide motifs. The maximum number of interrupting nucleotides  
230 in a compound SSR was set as 100. The statistics of SSRs (di- up to hexa-nucleotide)  
231 in the *A. duranensis* genome was shown in **Table S18**. In total, we detected 105,003  
232 SSRs in *A. duranensis* from which 84,464 SSR primers were designed. The di-  
233 nucleotide motif was the most abundant type and accounted for 43.45% of all SSRs,  
234 followed by tri-nucleotide (30.54%). In di-nucleotide type, AT motif was the most  
235 abundant type. In tri-nucleotide type, AAT was dominant (**Dataset S8**).

#### 236 **3.2 Single nucleotide polymorphism (SNPs)**

237 Reads from six re-sequenced genotypes including two A-genome genotypes (ICG  
238 8123 and ICG 8138) and four B-genome genotypes (ICG 8960, ICG 8209, ICG 13160  
239 and ICG 8206) (**Table S19**) were aligned to the reference genome using the Burrows-  
240 Wheeler Aligner program (BWA)<sup>37</sup>. About 70% of reads of A genomes (ICG 8123 and  
241 ICG 8138) could be mapped to the *A. duranensis* genome with a threshold that five  
242 mismatches are allowed, while ~45% of reads of B genomes (ICG 8960, ICG 8209,

243 ICG 13160 and ICG 8206) could be mapped (**Dataset S10**). SAMtools<sup>38</sup> (version 1.1)  
244 was used to call SNPs (**Table S20**). We identified 8,617,722-8,653,808 SNPs against  
245 A-genome genotypes and 3,684,730-3,884,005 against B-genome genotypes (**Table**  
246 **S20; Dataset S11**). Fewer SNPs were detected in B-genome genotypes due to fewer  
247 mapped reads. Structural variations such as insertions, deletions, copy number  
248 variations and inversions for the A- , B- and AB genomes were also identified (**Table**  
249 **S21**).  
250

#### 251 **4. Speciation of peanut A and B subgenome**

252 By performing a trio comparison of the synthetic tetraploid ISATGR 184 and its  
253 parents, ICG8123 and ICG8206, we studied the divergence between the subgenomes  
254 A and B. Parental reads were mapped to the reference genome and identified SNV  
255 between the two parental lines. In total, ~43% of reads from two parental lines were  
256 mapped to the reference genome. We filtered the SNPs by read coverage (>4x) and  
257 likelihood of second most likely genotype < 0.05. A total of 847676 high quality  
258 SNVs were identified between the two parental lines, meaning a mutation rates ~4.5 x  
259 10<sup>-4</sup> mutations at a base site in each line. Then, we mapped reads from ISATGR to the  
260 reference genome. In total, 76.04% of reads were successfully mapped. Genotypes are  
261 filtered by read coverage (>20x) and likelihood of second most likely genotype <  
262 0.05. We identified 748802 SNV sites between the two parental line and they were  
263 genotyped in the tetraploid species.  
264

#### 265 **5. Evolutionary analysis**

266 The phylogenetic tree was constructed using single-copy orthologous genes shared by  
267 *A. duranensis* and fifteen other plant species (soybean, *Medicago*, *Lotus*, pigeonpea,  
268 chickpea, common bean, canola, cotton, castor, linseed, *Arabidopsis*, apple, poplar,

269 tomato and rice) using the maximum-likelihood algorithm implemented in MEGA<sup>39</sup>.  
270 Colinear genes from *Medicago*<sup>40</sup>, soybean<sup>41</sup>, and grape<sup>42</sup> were used to locate related  
271 evolutionary events. We found evidence that peanut was affected by one lineage-  
272 specific event after its divergence from the *Medicago*-soybean lineage. Colinear genes  
273 within a genome and between different genomes were inferred by using MCScanX<sup>43</sup>.  
274 We adopted soybean genes' CDS in colinearity in its genome to search against peanut  
275 scaffold sequences to find best matching pairs of regions > 120 bp in length. Soybean  
276 genes were preferred over *Medicago* genes as reference to retrieve peanut homologs  
277 in that *Medicago* genes seem to accumulate mutations faster<sup>40</sup>. Genes with tandem  
278 duplicates in their respective neighboring 100 kb regions in soybean or from large  
279 gene families (with more than 30 genes at BLASTP E-value 1e-10) were removed  
280 from the present analysis. We inferred synonymous substitution rates between  
281 homologous genes by using the Nei-Gojobori approach implemented in PAML<sup>44</sup>.  
282 Peanut coding sequences were aligned with their soybean homologs codon by codon,  
283 estimating synonymous substitution rates ( $K_s$ ) between peanut and soybean homologs  
284 and between two retrieved peanut CDS. Accordingly,  $K_s$  between homologs within  
285 and among three other plants were estimated. The  $K_s$  distribution of peanut homologs  
286 shows a very prominent peak around  $K_s = 0.02-0.04$  (**Fig. 2d**), which suggests a  
287 peanut-specific polyploidization. Compared to a previously inferred soybean-specific  
288 polyploidization at ~13 Mya<sup>41</sup>, the peanut-specific event is much more recent,  
289 occurring ~5 Mya.

290 Reads from different genotypes were aligned to the reference genome by BWA<sup>37</sup>.  
291 SAMtools<sup>38</sup> (v1.1) were used to call single nucleotide variations (SNV). SNV sites  
292 were compared between parental lines and subgenomes in tetraploids to find likely  
293 converted sites and other mutated sites as previously described<sup>45</sup>. SNVs are identified  
294 between the two parental lines by mapping reads to the reference genome, with 72.0%  
295 and 43.1% of reads from ICG 8123 and ICG 8206 mapped respectively. We filtered  
296 SNVs by read coverage (>4x) and likelihood of second most likely genotype < 0.05. A

297 total of 847,676 high quality SNVs were identified between the two parental lines.  
298 About 76.04% of the reads from ISATGR 184 are mapped to the reference genome.  
299 Genotypes are filtered by read coverage (>20x) and likelihood of second most likely  
300 genotype < 0.05. A total of 748,802 SNV sites between the two parental lines were  
301 genotyped in the tetraploid species with high accuracy. We found that extensive gene  
302 conversion has taken place virtually immediately following polyploid formation, i.e.  
303 in the ~3 seed to seed generations that have passed following formation of this  
304 neopolyploid by human hands.

305

## 306 **6. Synteny analysis**

307 Promer package of MUMmer<sup>46</sup> was used to look for Maximal Unique Matches  
308 (MUMs) for the amino acid sequences aligned. The whole genome dot plots for these  
309 matches were depicted using the Mummerplot and gnuplot 4.4 patch level 2. The  
310 protein sequences of the genomes were compared and clustered using Vmatch<sup>47</sup> with  
311 a query and subject coverage of 85 % and 70 % respectively with a minimum match  
312 length of 100 and an exdrop of 100. Yn00 of PAML package was used for the  
313 identification of duplicated genes in the clusters. The matches were then further  
314 provided to i-ADHoRe<sup>48</sup> for the identification of syntenic blocks between two  
315 genomes. The coordinates of the first and last gene from these syntenic blocks were  
316 used for the construction of the Circos<sup>49</sup> image. The synonymous substitution rates  
317 between homologous genes were inferred using Nei Gojobori approach implemented  
318 in PAML<sup>44</sup>.

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323 **7. Genes involved in subterranean fructification, oil biosynthesis and**  
324 **encoding allergens.**

325 **7.1 Genes involved in gravitropism and photomorphogenesis**

326 In order to identify the genes involved in gravitropism in *A. duranensis*, a total of 162  
327 genes falling into the GO category “gravitropism” (GO:0009630) and 36 genes  
328 identified in *Arabidopsis* were extracted from proteome of *Arabidopsis* and searched  
329 against the *A. duranensis* gene set using Blastp with an E-value cutoff of 1e-10. The  
330 Blastp hits are then filtered based on 80% query coverage. Of the 198 gravitropism  
331 related genes, 137 had homologs in *A. duranensis*. The unidentified gravitropism-  
332 related genes is likely due to absence or mis-annotation of the *A. duranensis* genome.  
333 Further analysis based on previous functional studies<sup>50-62</sup> identified 24 *A. duranensis*  
334 genes likely to be gravitropic including 4 involved in gravity perception, 8 in signal  
335 transduction and 12 in organ response (**Dataset S15**). To identify  
336 photomorphogenesis-related genes in *A. duranensis*, a total of 280 genes related to  
337 photomorphogenesis identified in *Arabidopsis* were found to have 137 *A. duranensis*  
338 homologs using Blastp with an E-value cutoff of 1e-10. The values of Ka and Ks and  
339 the  $\omega$  (Ka/Ks) were estimated between homologous genes using Nei-Gojobori  
340 approach implemented in PAML<sup>44</sup>.

341 **7.2 Genes involved in oil biosynthesis**

342 Genes involved in oil biosynthesis in *Arabidopsis*  
343 (<http://aralip.plantbiology.msu.edu/downloads>) were retrieved from *Arabidopsis*  
344 proteome and searched (BLASTP E-value 1e-5) against soybean and peanut  
345 proteomes, independently. The resulting hits obtained from soybean and peanut were  
346 then mapped back to the categories as in the aralip database to obtain numbers.

347

348

### 349 7.3 Allergen-encoding genes

350 To date, at least 11 potential allergen proteins (Ara h 1-11) have been officially  
351 recognized by the International Union of Immunological Societies (IUIS,  
352 <http://www.allergen.org/Allergen.aspx>, last accessed December 12, 2014). These  
353 proteins were downloaded from GenBank and subjected to BLASTp analysis against  
354 the *A. duranensis* gene set with an E-value cutoff of 1e-30. Of the 11 allergens, nine  
355 were found in *A. duranensis*. Of the remained two allergens, the Ara h 6 was  
356 identified with an E-value cutoff of 1e-20, the other one (Ara h 4) has been renamed  
357 as Ara h 3. All known peanut allergens were identified in *A. duranensis* with an E  
358 value cutoff of 1e-20. In order to identify novel allergen-encoding genes in *A.*  
359 *duranensis*, 61 allergen proteins from other crops, like wheat, soybean and tomato,  
360 were also downloaded from IUIS. We searched for *A. duranensis* genes orthologous to  
361 these allergen-encoding genes, and identified 21 putative orthologs including 13  
362 potential novel allergen-encoding genes as well as 7 orthologs of known peanut  
363 allergen genes (**Dataset S16**). For further annotation, these genes were subject to  
364 similarity search against the Pfam database (<http://pfam.xfam.org/> last accessed  
365 December 13, 2014) with an E-value cutoff of 1e-5. These allergen-encoding genes  
366 were classified in 14 Pfam families, of which four families contain at least two genes.  
367 It is worth to note that Ara h 8 has three paralogs in the *A. duranensis* genome, and the  
368 identity between the paralogs ranging from 92~94%.

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## SI Tables:

**Table S1. Construction of libraries, generation and filtering of sequencing data used for genome assembly**

Platform	Library	Read Count	Average read length (bp)	Raw data (bp)	Sequence depth
<b>Illumina</b>	250 bp	138,068,824	125	34,517,206,000	25.01
	500 bp	137,054,823	125	34,263,705,750	24.83
	800 bp	115,096,083	125	28,774,020,750	20.85
	2000 bp	71,225,552	125	17,806,388,000	12.90
	5000 bp	91,106,606	125	22,776,651,500	16.50
	10000 bp	61,580,712	125	15,395,178,000	11.16
	20000 bp	305,601,609	125	76,400,402,250	55.36

**Table S2. Summary of the *A. duranensis* genome assembly**

	Contigs		Scaffolds	
	Size	Number	Size	Number
N90	5,864	36,381	148,975	1,718
N80	11,725	24,839	264,326	1,197
N70	17,450	18,084	376,360	864
N60	23,279	13,268	500,641	619
N50	29,584	9,555	649,840	437
Longest (bp)	285,529		5,342,956	
Total size (bp)	972,902,491		1,051,523,805	
Total number ( $\geq 100$ bp)		90,568		8,173
Total number ( $\geq 1$ kb)		67,603		5,025
Total number ( $\geq 2$ kb)		54,773		3,996



**Table S3. Distribution of contig and scaffold length for *A. duranensis* genome**

Contig					Scaffold			
Length (kb)	Number	Average length (bp)	Subtotal length (Mb)	Percentage (%)	Number	Average length (bp)	Subtotal length (Mb)	Percentage (%)
≥100	387	125,938	48.74	5.01	2,084	475,715	991.4	94.28
≥50	3,552	72,807	258.6	26.58	2,544	403,017	1,025	97.50
≥30	9,339	51,402	480.0	49.34	2,799	369,896	1,035	98.46
≥20	15,749	40,468	637.3	65.51	3,003	346,451	1,040	98.94
≥10	27,471	29,373	806.9	82.94	3,356	311,461	1,045	99.40
≥2	54,773	17,190	941.6	96.78	3,996	262,271	1,048	99.67
≥1	67,619	14,197	959.9	98.67	5,027	208,754	1,049	99.80

**Table S4. Assessment of the assembled genome through PCR amplification**

<b>Category</b>	<b>Fragment number</b>
Total primer pairs used	411
Number of amplified primers	365
Number of non-amplified primers	46
Primers with single amplified fragment	264
Primers with multiple amplified fragments	101
Primers with major amplified fragment	50

**Table S5. Evaluation of completeness of the genome assembly using core eukaryotic gene mapping approach (CEGMA)**

<b>Parameter</b>		<b>Number</b>	<b>Percent (%)</b>
Total KOGs		458	
One KOG align one gene		410	89.52
One KOG align one gene	overlap>0.7	370	80.78
	overlap >0.5	404	88.21
One KOG align several genes		31	6.76
One KOG align no gene		17	3.71

**KOGs=Eukaryotic orthologous gene sequences**

**Table S6. Assessment of gene space captured in genome assembly using all libraries**

	<b>Illumina PE Reads</b>	<b>Illumia MP Reads</b>	<b>454 Reads</b>
Total Reads	781,795,634.00	565,857,140	48,433,168
Mapped reads	688,385,989.00	480,729,955	47,943,412
Mapping percentage (%)	88.05%	84.97%	98.99
Genome coverage at $\geq 1x$ (%)	84.68%	63.56%	85.42
Genome coverage at $\geq 2x$ (%)	83.00%	60.55%	83.76
Genome coverage at $\geq 5x$ (%)	78.14%	54.08	77.65
Genome coverage at $\geq 10x$ (%)	70.34%	44.485	63.77
Genome coverage at $\geq 15x$ (%)	61.79%	35.875	47.16
Bases not covered (bp)	326,410,883	775,155,378	157,073,491
% of bases not covered	15.15	33.98	14.58
Average depth	31.97	21.56	22.87
Total bases (bp)	2,150,737,583	2,150,841,427	1,077,216,168

**Table S7. Estimation of *A. duranensis* genome using K-mer statistics**

<b>K-mer value</b>	<b>K-mer number</b>	<b>Depth</b>	<b>Genome size (bp)</b>	<b>Used bases</b>	<b>Used reads</b>	<b>Depth (X)</b>	<b>Average read length (bp)</b>
25	60,198,113,206	24	1,381,794,909	78,961,359,034	781,793,678	57.14	101

**Table S8. Gene sets used in this study from different plant species**

Species	Database	Version
Soybean	Phytozomev9.1	JGI Glyma1.1 annotation of the chromosome-based Glyma1 assembly
Medicago	Phytozomev9.1	Mt3.5v4 on assembly MedtrA17_3.5 from the Medicago Genome Sequence Consortium
Lotus	kazusa.or.jp	lotus_r2.5
Common bean	Phytozomev9.1	JGI annotation v1.0 on assembly v1.0 using published ESTs, and JGI RNAseq
Chickpea	Legume Information System	v1.0
Pigeonpea	Legume Information System	v1.0
Canola	Phytozomev9.1	BrapaFPsc_277_v1.3
Cotton	Phytozomev9.1	JGI annotation v2.1 on assembly v2.0
Castor	Phytozomev9.1	TIGR release 0.1
Linseed	Phytozomev9.1	Lusitatissimum_200_v1.0
Arabidopsis	Phytozomev9.1	TAIR release 10 acquired from TAIR
Apple	Phytozomev9.1	GDR prediction v1.0 on Malus x domestica assembly v1.0
Poplar	Phytozomev9.1	JGI assembly release v3.0, annotation v3.0
Tomato	Phytozomev9.1	SGNTomato Genome Project ITAG2.3
Rice	Phytozomev9.1	MSU Release 7.0 of the Rice Genome Annotation

**Table S9. The statistics of aligned genes between *A. duranensis* and other plant species with an E value cutoff of 1e-5.**

Species	<i>A. duranensis</i>		Aligned species	
	Matched genes	Percentage	Matched genes	Percentage
<i>A. duranensis</i> vs Arabidopsis	22132	43.98	13185	48.09
<i>A. duranensis</i> vs Canola	23129	45.96	13973	34.51
<i>A. duranensis</i> vs Chickpea	24496	48.68	14116	49.93
<i>A. duranensis</i> vs Pigeonpea	31456	62.51	16570	34.04
<i>A. duranensis</i> vs Soybean	26836	53.33	17445	31.13
<i>A. duranensis</i> vs Cotton	22990	45.68	15733	41.95
<i>A. duranensis</i> vs Lotus	27400	54.45	13079	33.99
<i>A. duranensis</i> vs Linseed	21798	43.32	14082	32.39
<i>A. duranensis</i> vs Apple	23827	47.35	14468	22.78
<i>A. duranensis</i> vs Medicago	28831	57.29	16081	31.60
<i>A. duranensis</i> vs Rice	22572	44.85	12224	30.07
<i>A. duranensis</i> vs Poplar	23899	47.49	15840	38.32
<i>A. duranensis</i> vs Common bean	26978	53.61	15391	56.59
<i>A. duranensis</i> vs Castor	25245	50.16	13689	43.85
<i>A. duranensis</i> vs Tomato	23640	46.98	13205	38.03

**Table S10. General statistics of predicted protein-coding genes in *A. duranensis* and comparison with other plant species**

Gene set	Common name	Number of genes	Average gene length (bp)	Average CDS length (bp)	Average exon per gene	Average intron length (bp)
<b>Reference</b>	<i>A. duranensis</i>	50,324	3,057.92	312.36	3.37	709.57
	Soybean	56,044	4,671.51	214.91	10.22	486.85
	Medicago	50,894	3,064.99	231.70	5.87	413.13
	Lotus	38,482	1,494.66	258.73	2.96	447.89
	Common bean	27,197	4,048.62	234.25	6.85	449.59
	Chickpea	28,269	3,055.39	236.51	4.93	448.78
	Pigeonpea	48,680	2,348.70	267.39	3.59	458.45
	Canola	40,492	2,274.32	230.81	5.63	185.02
<b>Homology</b>	Cotton	37,505	3,914.53	203.66	14.07	333.79
	Castor	31,221	2,261.54	242.46	4.17	339.80
	Linseed	43,471	2,307.97	238.58	5.03	260.97
	Arabidopsis	27,416	2,335.51	220.87	7.57	150.06
	Apple	63,514	2,639.37	236.13	4.74	383.85
	Poplar	41,335	3,759.28	211.44	11.13	359.97
	Tomato	34,727	3,163.56	228.78	4.62	505.12
	Rice	40,648	3,169.63	240.49	5.90	370.11

#Protein sequences from 15 sequenced plant species were used to perform gene prediction, taking one species each time. We mapped them to the genome assembly using TblastN (E-value- 1e-5). After this, homologous genome sequences were aligned against the matching proteins for accurate spliced alignments.



**Table S11. Functional annotation of predicted genes in *A. duranensis***

<b>Database</b>	<b>Number</b>	<b>Percentage</b>
SWISS-PROT	20,701	41.13 %
TrEMBL	35,365	70.27 %
NR	35,726	70.99%
NT	40,552	80.58%
InterPro	30,032	59.68 %
KEGG	30,573	60.75 %
GO	24,498	48.68 %
Pfam	25,771	51.21 %
CDD	23,903	47.50 %
Un-annotated	5,494	10.9%

**Table S12. Details on gene family for *A. duranensis* and other plant species**

<b>Species</b>	<b>Total predicted genes</b>	<b>Genes in orthologous groups</b>	<b>Genes not in orthologous groups<sup>1</sup></b>	<b>Total orthologous groups<sup>2</sup></b>	<b>Species-specific homolog groups<sup>3</sup></b>	<b>Average genes group</b>
<i>A. duranensis</i>	50,324	40,736	9,588	14,005	1,423 (16,472)	2.91
Chickpea	31,988	30,412	1,576	14,657	348 (1,375)	2.07
Pigeonpea	48,680	42,353	6,327	17,222	1,440 (7,934)	2.46
Soybean	73,320	62,797	10,523	17,900	1,265 (3,331)	3.51
Medicago	45,888	32,786	13,102	14,159	2,202 (9,533)	2.32
Lotus	42,399	24,345	18,054	14,599	1,155 (4,248)	1.67
Common bean	31,638	29,666	1,972	15,908	271 (801)	1.86
Linseed	43,484	37,033	6,451	14,258	1,474 (4,907)	2.60
Canola	101,040	81,965	19,075	21,752	5,582 (17,934)	3.77
Castor	31,221	21,077	10,144	15,360	604 (1,608)	1.37
Cotton	77,267	71,534	5,733	16,910	2,016 (6,652)	4.23
Rice	49,061	36,506	12,555	13,995	2,900 (10,795)	2.61
Tomato	34,727	26,231	8,496	14,260	983 (3,873)	1.84
Apple	63,517	48,160	15,357	17,200	3,659 (11,308)	2.80
Arabidopsis	35,386	31,882	3,504	16,329	577 (1,550)	1.95
Poplar	73,013	64,680	8,333	16,465	1,475 (4,410)	3.93

<sup>1</sup>Predicted genes that were not organized into groups using OrthoMCL. We suggest that many such genes are misannotated, though we cannot rule out genes with unique domain arrangements that have undergone lineage specific expansion. <sup>2</sup>Orthologous groups containing at least one gene from the indicated species. <sup>3</sup>Groups containing putative paralogs from the indicated species, but lacking genes from other species. Such unassigned homologous groups may contain genes with ambiguous relationships among species, such as many of the NBS-LRR disease resistance genes that can evolve by processes such as non-allelic recombination and gene conversion.

**Table S13. Comparison of *A. duranensis* gene families with soybean and *Medicago***

Family size	Difference in gene copy number															
	<-6	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	>6	Total
<b>Shared gene families between <i>A. duranensis</i> and Soybean</b>																
1								94.66	4.49	0.31	0.08	0.08	0.02	0.36	0.34	6197
2							85.43	12.9	1.07	0.25	0.13	0.03	0.03	0.09	0.06	3170
3						67.87	25.11	5.48	0.88	0	0	0	0.11	0.22	0.22	912
4				48.89	31.67	15.56	2.5	0	0	0	0	0	0	0.28	0.28	360
5			34.01	34.52	19.29	6.09	2.54	0	0.51	0	0	0	0	0.51	0.51	197
6		33.66	34.65	17.82	6.93	0.99	0	0	0	0	0	0	0	0	0	101
7		8.57	31.43	25.71	11.43	10	2.86	0	0	0	0	0	0	0	0	70
8	19.35	12.9	16.13	11.29	9.68	9.68	1.61	1.61	1.61	1.61	0	0	0	1.61	1.61	62
9	22.73	25	13.64	6.82	6.82	2.27	2.27	0	0	0	0	0	0	0	0	44
10	33.33	2.56	15.38	12.82	2.56	5.13	0	2.56	0	0	0	0	0	0	0	39
<b>Shared gene families between <i>A. duranensis</i> and <i>Medicago</i></b>																
1								90.34	8.33	0.77	0.22	0.08	0	0.24	0.22	6325
2						67.24	24.14	6.65	0.99	0.25	0	0	0	0.57	0.57	1218
3					49.86	24.23	16.06	4.23	2.25	1.13	0.28	0.28	0.85	0.85	355	
4				30.67	28	20	10	4.67	1.33	1.33	0	0	1.33	1.33	150	
5			33.72	16.28	16.28	9.3	5.81	5.81	2.33	1.16	1.16	0	2.33	1.16	86	
6		26.79	16.07	17.86	10.71	8.93	3.57	1.79	3.57	0	0	0	0	0	56	
7		16.67	9.52	7.14	9.52	11.9	11.9	9.52	4.76	2.38	0	0	0	0	42	
8	16.67	23.33	6.67	3.33	6.67	6.67	0	3.33	0	0	3.33	0	0	3.33	3.33	30
9	37.04	3.7	7.41	3.7	0	3.7	3.7	3.7	0	0	3.7	0	0	0	0	27
10	25	3.57	3.57	7.14	0	3.57	3.57	3.57	7.14	0	3.57	3.57	0	0	0	28

**Table S14. Details on single copy orthologs and unique paralogs in *A. duranensis* and other plant species**

<b>Species</b>	<b>Single-copy orthologs</b>	<b>Co-orthologs<sup>1</sup></b>	<b>Unique paralogs</b>	<b>Other orthologs<sup>2</sup></b>	<b>Unclustered genes</b>
<i>A. duranensis</i>	9,968	7,138	16,472	7,158	9,588
Chickpea	8,346	12,125	1,375	8,566	1,576
Pigeonpea	11,022	11,201	7,934	12,196	6,327
Soybean	3,779	25,189	3,331	30,498	10,523
Medicago	7,929	9,403	9,533	5,921	13,102
Lotus	10,120	7,931	4,248	2,046	18,054
Common bean	9,791	11,609	801	7,465	1,972
Linseed	2,895	14,369	4,907	14,862	6,451
Canola	1,531	25,579	17,934	36,921	19,075
Castor	12,258	7,400	1,608	189	10,144
Cotton	4,808	29,975	6,652	30,099	5,733
Rice	5,654	12,793	10,795	7,264	12,555
Tomato	9,247	9,151	3,873	3,960	8,496
Apple	4,710	15,529	11,308	16,613	15,357
Arabidopsis	9,293	11,947	1,550	9,092	3,504
Poplar	3,674	26,347	4,410	30,249	8,333

<sup>1</sup>Co-orthologous genes, also known as “in-paralogs”, are derived from duplication in the indicated genome. <sup>2</sup>Other orthologs represent gene duplication events internal to the overall set, but basal more than two of the compared species.

**Table S15. Summary of predicted non-protein coding genes in *A. duranensis* genome**

Type	Sub-type	Number	Average length (bp)	Total length (bp)	Percent (%)
miRNA		816	107.34	87,598	0.0063
tRNA		913	73.28	66,904	0.0048
rRNA	5S rRNA	61	116.59	7,112	0.0005
	5.8S rRNA	17	152.94	2,600	0.0002
	18S rRNA	21	1944.67	40,838	0.0029
	28S rRNA	16	4579.94	73,279	0.0053
	Total rRNA	115	1076.77	123,829	0.0089
snRNA	CD- box snRNA	71	106.73	7,578	0.0005
	Splicing snRNA	131	137.85	18,058	0.0013
	Total snRNA	202	126.91	25,636	0.0018

**Table S16. New miRNAs identified in the *A. duranensis* genome**

<b>ID</b>	<b>Sequence</b>	<b>Length</b>	<b>Scaffold</b>	<b>Start</b>	<b>End</b>	<b>Strand</b>
Peanut_m0002-3p	ATAACCAAGGAAAAGACATT	20	scaffold1297	106541	106560	-
Peanut_m0003-3p	ACTTAGGCCTTAGAACTTAT	20	scaffold18250	900	919	+
Peanut_m0004-3p	ACATTAAACATGGGACAATTTA	22	scaffold1988	30519	30540	+
Peanut_m0007-3p	TGAGATATCTCTTCCAGAAG	20	scaffold371	58057	58076	-
Peanut_m0009-3p	GACTGTAGAGTGGTAATTCAA	21	scaffold426	160999	161019	-
Peanut_m0014-3p	ACAGCCATTTTTGCCGAGTT	20	scaffold918	204369	204388	-
Peanut_m0001-5p	CAGGAGACCCGGGTTCGATTCCC	23	scaffold1221	110014	110036	+
Peanut_m0005-5p	CTTTAGGTCAATGATTGGTA	20	scaffold2433	93926	93945	-
Peanut_m0006-5p	AGTTCTGAGAAGTCTTCTTTG	21	scaffold3536	27272	27292	-
Peanut_m0008-5p	AGAAGAACTTGTAGGTGTTGAA	22	scaffold4210	29738	29759	-
Peanut_m0010-5p	GAGGAGACAGAAACAGGTAG	20	scaffold454	183899	183918	-
Peanut_m0011-5p	TGACTTTTGGAAAATGTTTG	20	scaffold495	204813	204832	+
Peanut_m0012-5p	TTCTGACTTCTTTAGGCAGT	20	scaffold6457	39441	39460	+
Peanut_m0013-5p	TCTCTGCAGAAGGAATGACA	20	scaffold681	121285	121304	-
Peanut_m0015-5p	GTGCAGGACGATGTCGTTGC	20	scaffold9422	15413	15432	+

**Table S17. Target genes and their function annotation of new miRNAs in *A. duranensis***

<b>miRNA ID</b>	<b>Number of target genes</b>	<b>CDD (Conserved Domains Database)</b>	<b>Putative functions of target genes</b>
m0001-5p	3	COG1691	NCAIR mutase (PurE)-related proteins
		PLN03195	fatty acid omega-hydroxylase
		TIGR03225	benzoyl-CoA oxygenase, B subunit
m0002-3p	17	cd00303	Retropepsins
		cd11236	MET-like receptor tyrosine kinases
		COG3083	Predicted hydrolase of alkaline phosphatase superfamily
		COG4036	Predicted membrane protein
		COG5222	Uncharacterized conserved protein, contains RING Zn-finger
		pfam04900	Fcf1
		PRK00232	4-hydroxythreonine-4-phosphate dehydrogenase
		PRK06599	DNA topoisomerase I
		PRK08377	NADH dehydrogenase subunit N
		PRK09330	cell division protein FtsZ
		PRK09629	bifunctional thiosulfate sulfurtransferase
		PRK12679	transcriptional regulator Cbl
		PRK13902	alanyl-tRNA synthetase
		TIGR04055	putative heme d1 biosynthesis radical SAM protein NirJ2
m0003-3p	1	TIGR01160	translation initiation factor SUI1, eukaryotic
m0004-3p	1	COG0061	NAD kinase
m0005-5p	1	PLN02393	leucoanthocyanidin dioxygenase like protein
m0006-5p	6	pfam09773	Meckelin (Transmembrane protein 67)

		pfam13639	Ring finger domain
		PRK13897	type IV secretion system component VirD4
		PTZ00350	adenylosuccinate synthetase
		smart00220	Serine/Threonine protein kinases, catalytic domain
m0007-3p	1	pfam03124	EXS family
m0008-5p	9	cd00180	Catalytic domain of Protein Kinases
		pfam05133	Phage portal protein, SPP1 Gp6-like
		pfam05297	Herpesvirus latent membrane protein 1 (LMP1)
		pfam06291	Bor protein
		PLN02499	glycerol-3-phosphate acyltransferase
		PRK04028	glutamyl-tRNA(Gln) amidotransferase subunit E
		PTZ00479	RAP Superfamily
		TIGR02168	chromosome segregation protein SMC, common bacterial type
		TIGR03981	His-Xaa-Ser system putative quinone modification maturase
m0010-5p	1	PLN02311	chalcone isomerase
m0011-5p	6	cd01851	Guanylate-binding protein (GBP) family (N-terminal domain)
		cd08866	Ligand-binding SRPBCC domain
		pfam00587	tRNA synthetase class II core domain (G, H, P, S and T)
		PHA02746	protein tyrosine phosphatase
		PLN03240	putative Low-temperature-induced protein
m0013-5p	2	pfam05699	hAT family dimerisation domain
		TIGR02169	chromosome segregation protein SMC
m0015-5p	1	COG1752	Predicted esterase of the alpha-beta hydrolase superfamily



**Table S18. Summary of simple sequence repeats in *A. duranensis* regarding their distribution and primer design for peanut genetics and breeding applications.**

<b>SSR Statistics</b>	<b>Numbers</b>
Total number of sequences examined	20,597
Total size of examined sequences (bp)	1,077,216,168
Total number of identified SSRs	105,003
Number of SSR containing sequences	15,209
Number of sequences containing more than 1 SSR	12,308
Number of SSRs present in compound formation	25,672
<b>Distribution to different repeat type classes</b>	
Number of di-nucleotide repeats	45,622
Number of tri-nucleotide repeats	32,070
Number of tetra-nucleotide repeats	3,966
Number of penta-nucleotide repeats	1,450
Number of hexa-nucleotide repeats	428
Number of compound repeats	21,467
<b>Primer pairs for SSRs</b>	
Scaffolds were used to design primer pairs	11,712
Total numbers of primer pairs designed	84,464

**Table S19. Details on re-sequencing data of ten genotypes including four synthetic tetraploids and six diploids**

<b>Germplasm</b>	<b>Ploidy (genome)</b>	<b>Parental combinations</b>	<b>Read type</b>	<b>Number of reads</b>	<b>Read length (bp)</b>	<b>Data size (bp)</b>
ISATGR_5	Synthetic tetraploid (BB)	[ <i>A. magna</i> (ICG 8960) x <i>A. batizocoi</i> (ICG 8209)]	Paired end	983,446,602	101	99,328,106,802
ISATGR_278-18	Synthetic tetraploid (AB)	[ <i>A. duranensis</i> (ICG 8138) x <i>A. batizocoi</i> (ICG 13160)]	Paired end	1,230,617,008	101	124,292,317,808
ISATGR_1212	Synthetic tetraploid (AB)	[ <i>A. duranensis</i> (ICG 8123) x <i>A. ipaensis</i> (ICG 8206)]	Paired end	914,091,908	101	92,323,282,708
ISATGR_184	Synthetic tetraploid (AB)	[ <i>A. ipaensis</i> (ICG 8206) x <i>A. duranensis</i> (ICG 8123)]	Paired end	1,258,898,410	101	127,148,739,410
ICG_8123	Diploid (A)	<i>A. duranensis</i>	Paired end	504,473,764	101	50,951,850,164
ICG_8138	Diploid (A)	<i>A. duranensis</i>	Paired end	503,836,436	101	50,887,480,036
ICG_8960	Diploid (B)	<i>A. magna</i>	Paired end	461,986,170	101	46,660,603,170
ICG_8209	Diploid (B)	<i>A. batizocoi</i>	Paired end	458,037,642	101	46,261,801,842
ICG_13160	Diploid (B)	<i>A. batizocoi</i>	Paired end	487,100,820	101	49,197,182,820
ICG_8206	Diploid (B)	<i>A. ipaensis</i>	Paired end	553,199,484	101	55,873,147,884

**Table S20: Distribution of SNPs identified among the A genomes (two genotypes) and B genomes (four genotypes)**

	<u>ICG 8123</u>		<u>ICG 8138</u>		<u>ICG 8960</u>		<u>ICG 8209</u>		<u>ICG 13160</u>		<u>ICG 8206</u>	
	SNP	Rate	SNP	Rate	SNP	Rate	SNP	Rate	SNP	Rate	SNP	Rate
<b>Gene region</b>	1,437,202	16.677	1,438,084	16.618	1,243,501	33.479	1,280,304	32.964	1,262,785	34.271	1,274,376	32.868
<b>Exon</b>	453,740	5.265	450,314	5.204	423,103	11.391	429,687	11.063	428,290	11.623	436,853	11.267
<b>Intron</b>	968,333	11.237	972,585	11.239	807,968	21.753	838,142	21.579	822,277	22.316	824,558	21.266
<b>Others</b>	15,129	0.176	15,185	0.175	12,430	0.335	12,475	0.321	12,218	0.332	12,965	0.334
<b>ncRNA</b>	1,567	0.018	1,311	0.015	849	0.023	896	0.023	885	0.024	771	0.02
<b>tRNA</b>	253	0.003	229	0.003	71	0.002	67	0.002	81	0.002	55	0.001
<b>rRNA</b>	407	0.005	202	0.002	110	0.003	125	0.003	131	0.004	101	0.003
<b>snRNA</b>	248	0.003	240	0.003	153	0.004	190	0.005	186	0.005	146	0.004
<b>miRNA</b>	659	0.008	640	0.007	515	0.014	514	0.013	487	0.013	469	0.012
<b>TEs</b>	792,959	9.201	790,347	9.133	219,683	5.915	217,558	5.601	203,212	5.515	230,178	5.937
<b>Others</b>	6,385,994	74.103	6,424,066	74.234	2,250,204	60.583	2,385,247	61.412	2,217,848	60.19	2,371,974	61.176
<b>Total</b>	8,617,722	100	8,653,808	100	3,714,237	100	3,884,005	100	3,684,730	100	3,877,299	100

**Table S21: Summary of structural variations in diploid (A-genome and B-genome) and synthetic (AB-genome) genotypes**

	No. of SVs		Total length (kb)	Average length (bp)
<b>Diploid A genome</b>				
<b>Sample</b>	<b>PI 475845-reference genome</b>			
Insertion	0		0	0
Deletion	33,648		116,094.635	3,450.269
Inversion	3,003		55,763.004	18,569.099
CNVs	15,958	gain : 4,243	-	-
		loss : 11,715		
<b>Sample</b>	<b>ICG 8138</b>			
Insertion	0		0	0
Deletion	23,077		122,149.219	5,293.115
Inversion	2,234		46,606.732	20,862.458
CNVs	20,776	gain : 11,858	-	-
		loss : 8,918		
<b>Sample</b>	<b>ICG 8123</b>			
Insertion	0		0	0
Deletion	22,600		119,789.414	5,300.417
Inversion	2,084		43,681.400	20,960.365
CNVs	20,955	gain : 12,369	-	-
		loss : 8,586		
<b>Diploid A genome</b>				

<b>Sample</b>	<b>ICG 8960</b>		
Insertion	0		0
Deletion	8,946		60,149.980
Inversion	1,378		32,379.408
CNVs	24,132	gain : 13,288	-
		loss : 10,844	
<b>Sample</b>	<b>ICG 8209</b>		
Insertion	0		0
Deletion	9,723		61,424.708
Inversion	1,417		29,805.699
CNVs	24,146	gain : 10,729	-
		loss : 13,417	
<b>Sample</b>	<b>ICG 13160</b>		
Insertion	0		0
Deletion	10,344		61,214.867
Inversion	1,396		32,590.761
CNVs	24,188	gain : 9,559	-
		loss : 14,629	
<b>Sample</b>	<b>ICG 8206</b>		
Insertion	0		0
Deletion	9,801		64,806.025
Inversion	1,488		36,921.970
CNVs	24,092	gain : 11,957	-
		loss : 12,135	

<b>Synthetic genotypes</b>			
<b>Sample</b>	<b>ISATGR-5</b>		
Insertion	0	0	0
Deletion	15,149	87,538.429	5,778.496
Inversion	2,380	53,421.757	22,446.116
CNVs	24,434	gain : 18,733 loss : 5,701	-
<b>Sample</b>	<b>ISATGR 278-18</b>		
Insertion	0	0	0
Deletion	29,842	158,257.555	5,303.182
Inversion	3,662	76,232.457	20,817.165
CNVs	20,913	gain : 13,367, loss : 7,546	-
<b>Sample</b>	<b>ISATGR 1212</b>		
Insertion	0	0	0
Deletion	24,747	135,350.592	5,469.374
Inversion	3,184	68,492.371	21,511.423
CNVs	20,939	gain : 13,219 loss : 7,720	-
<b>Sample</b>	<b>ISATGR 184</b>		
Insertion	1	0.217	217
Deletion	31,651	168,895.231	5,336.174
Inversion	3,802	79,680.365	20,957.487
CNVs	20,601	gain : 12,384	-

**Table S22. Summary of putative acyl lipid genes in *A. duranensis*, *Arabidopsis* and soybean**

<b>Category of lipid genes</b>	<b><i>A. duranensis</i></b>	<b><i>Arabidopsis</i></b>	<b>Soybean</b>
Phospholipase	115	90	120
Miscellaneous lipid synthesis related	92	73	93
Sphingolipid synthesis	40	28	40
Phospholipid synthesis in mitochondria	16	10	16
Fatty acid synthesis in plastids	73	48	72
Aromatic suberin synthesis	14	8	14
Lipid signaling	187	142	191
Aliphatic suberin synthesis	42	34	42
Eukaryotic phospholipid synthesis	75	45	75
Lipase	330	269	336
Lipid trafficking	10	6	10
Cuticular wax synthesis	191	167	200
Mitochondrial fatty and lipoic acid synthesis	22	13	22
TAG degradation	47	35	47
TAG synthesis	96	68	96
Fatty acid elongation and cuticular wax synthesis	30	26	30
GDSL	127	106	127
Beta-oxidation	35	25	35
Lipid acylhydrolase	15	11	15
Galactolipid degradation	10	7	10
Cutin synthesis	31	28	31
Plastidial glycerolipid, galactolipid and sulfolipid synthesis	73	52	73
Total	1671	1291	1695

**Table S23. Summary of samples collected during seed development in peanut**

<b>Stages</b>	<b>Samples</b>	<b>Seed size (mm)</b>
P5	Seed	1.0 – 2.0
P6	Seed	2.0 – 4.0
P7	Seed	4.0 – 6.0
P8	Seed	6.0 – 8.0
P9	Seed	8.0 – 10.0
P10	Seed	10.0 – 12.0



## SI Figures:



**Figure S1.** *A. duranensis* accessions PI475845 (reference genome). The red arrows show the aerially developing pegs, and the red dash box shows the pods developed underground. Aerially pegs do not normally expand until penetration into the soil. This accession was collected from Tariji Bolivia (Latitude: 21.53, Longitude: 63.38) in 1977 by collectors GKBSPPSc (Gregory, W.C.; Krapovickas, A.; Banks, D.J.; Simpson, C.E.; Pietrarelli, J.; and Schinini, A.) (Stalker et al., 1995).

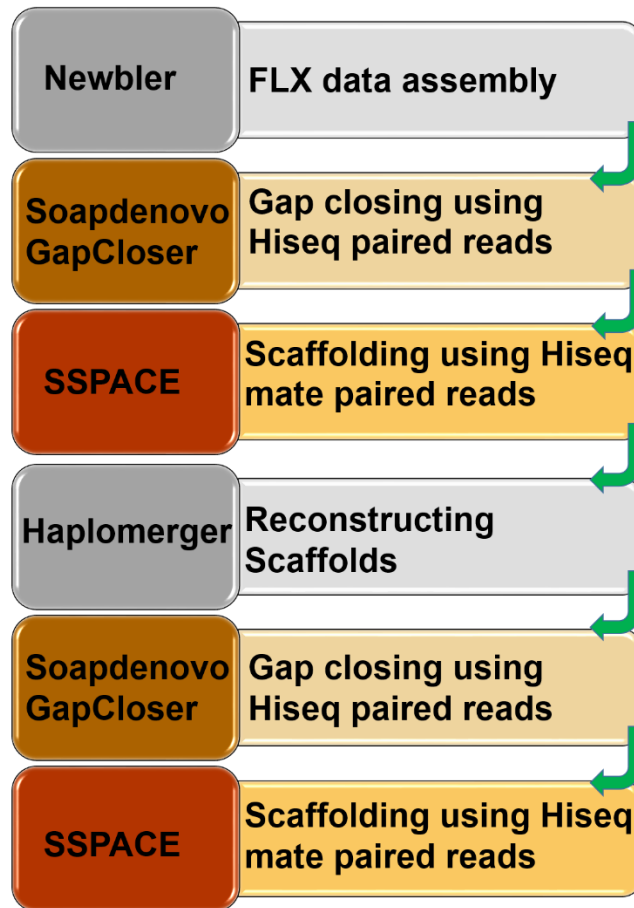
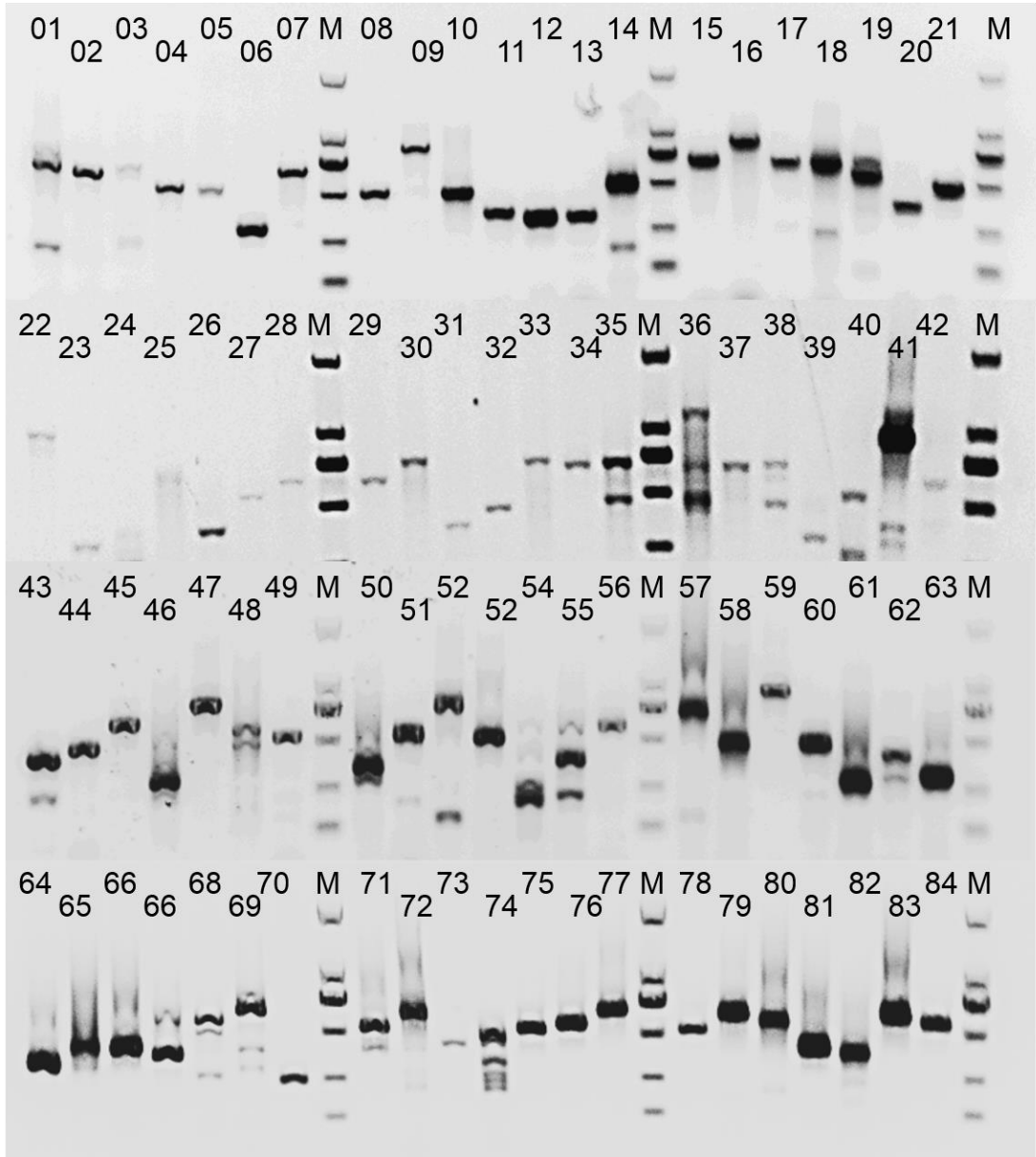
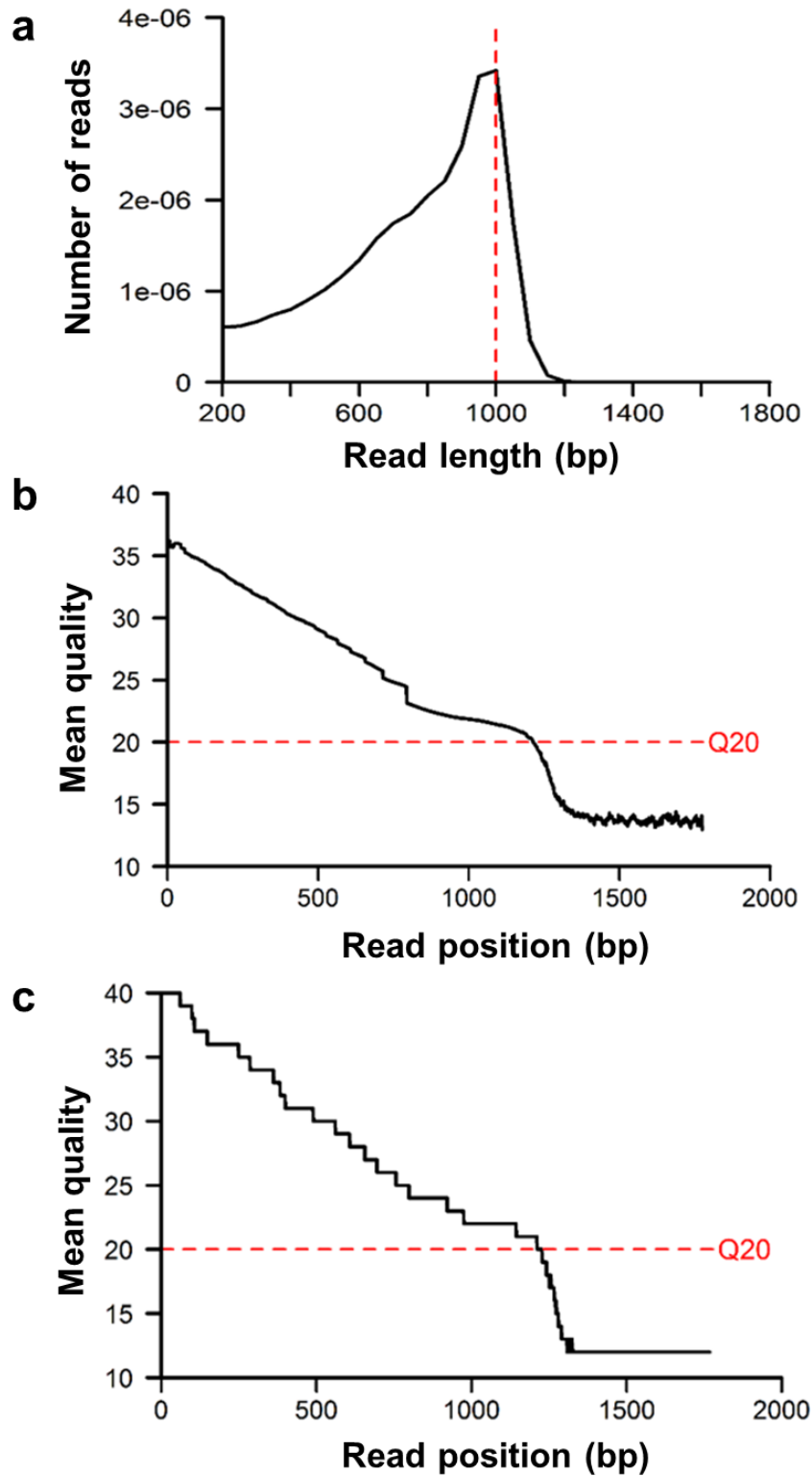


Figure S2. Flowchart of the approaches used for *de novo* assembly

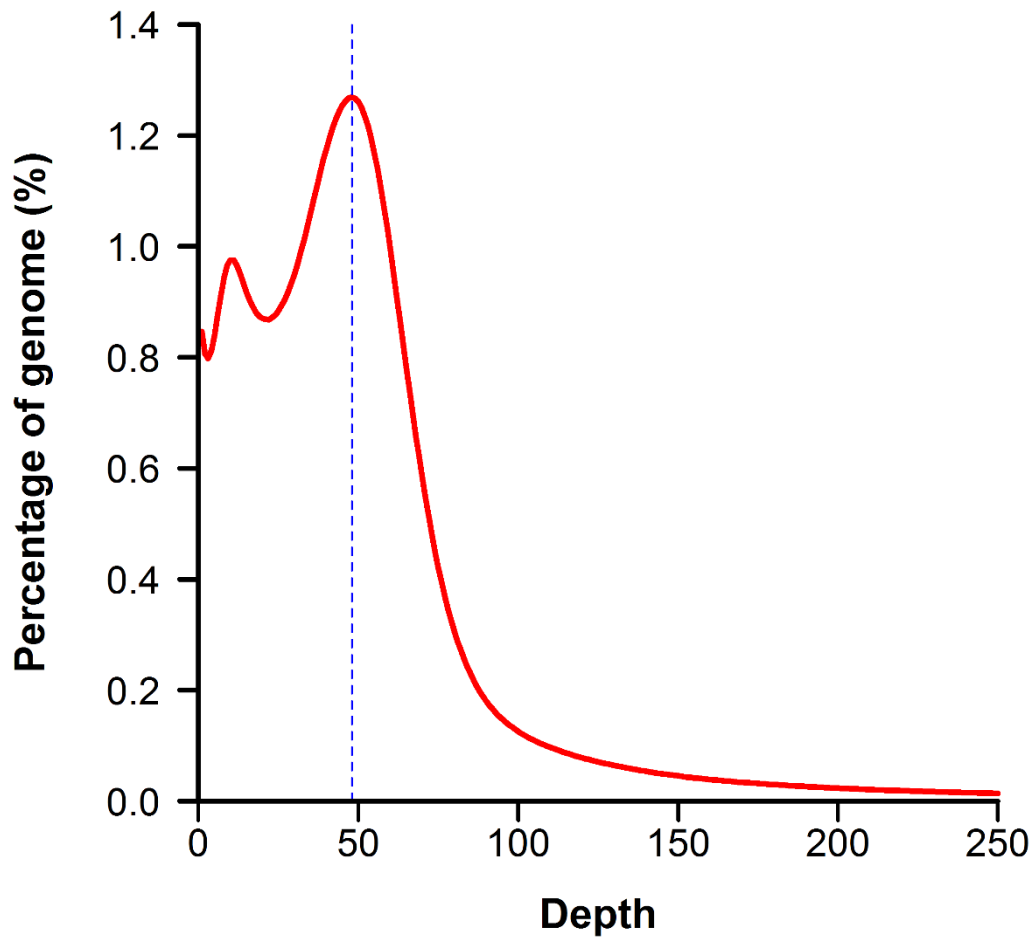


**Figure S3. Evaluation of the *A. duranensis* assembled genome using PCR amplification**

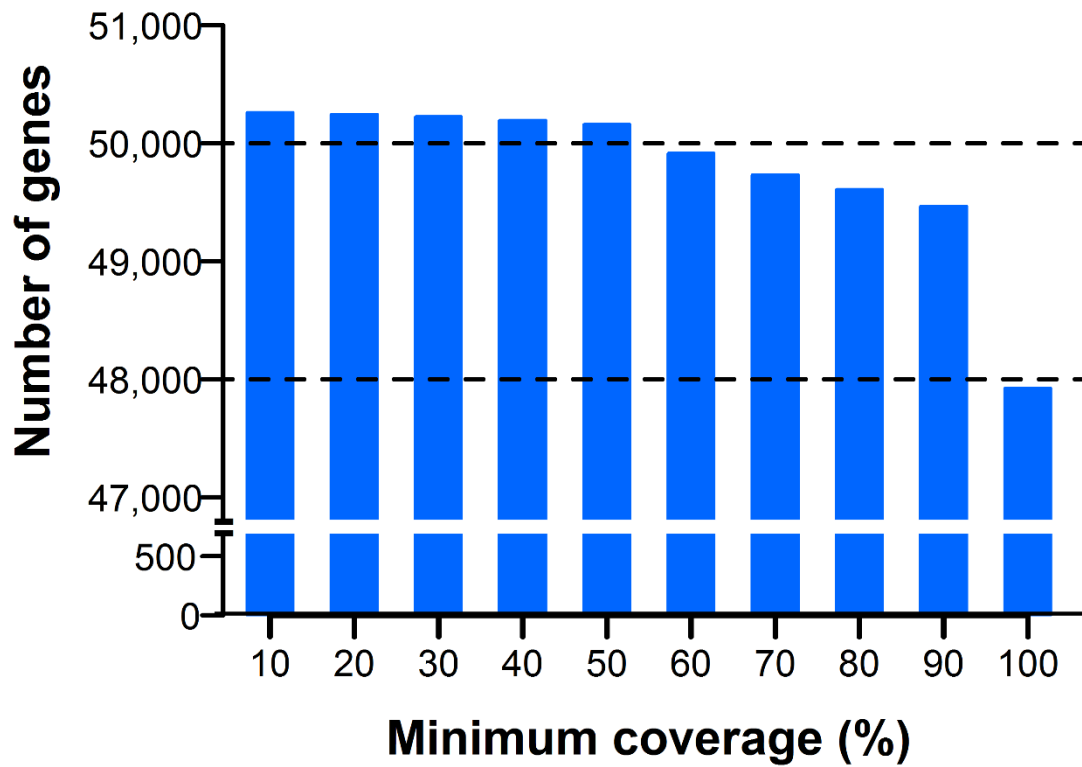


**Figure S4. Quality assessment of the sequencing data.**

The distributions were computed using FastQC (a) read length distribution, (b) mean read quality per read position, (c) median read quality per read position.

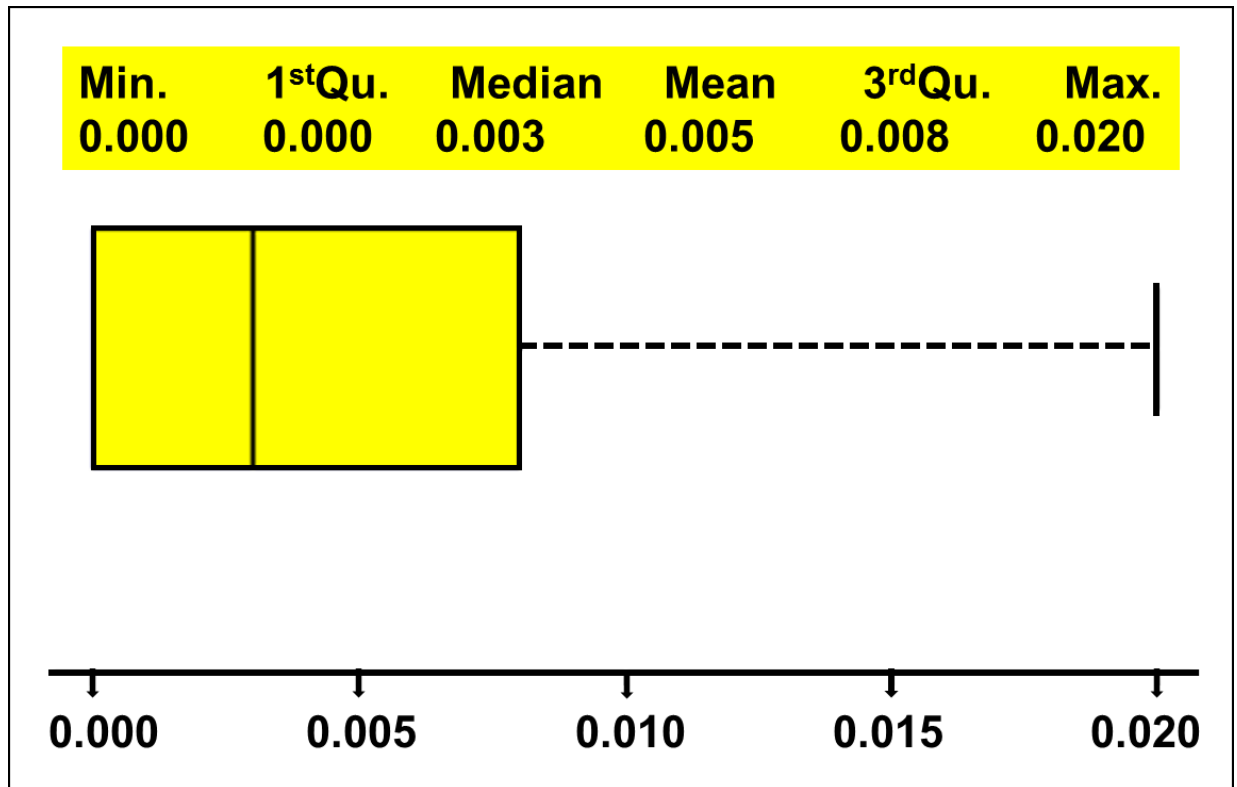


**Figure S5. Distribution of sequence depth across the assembled genome.**  
The Y-axis represents the proportion of the genome at a given sequencing depth.

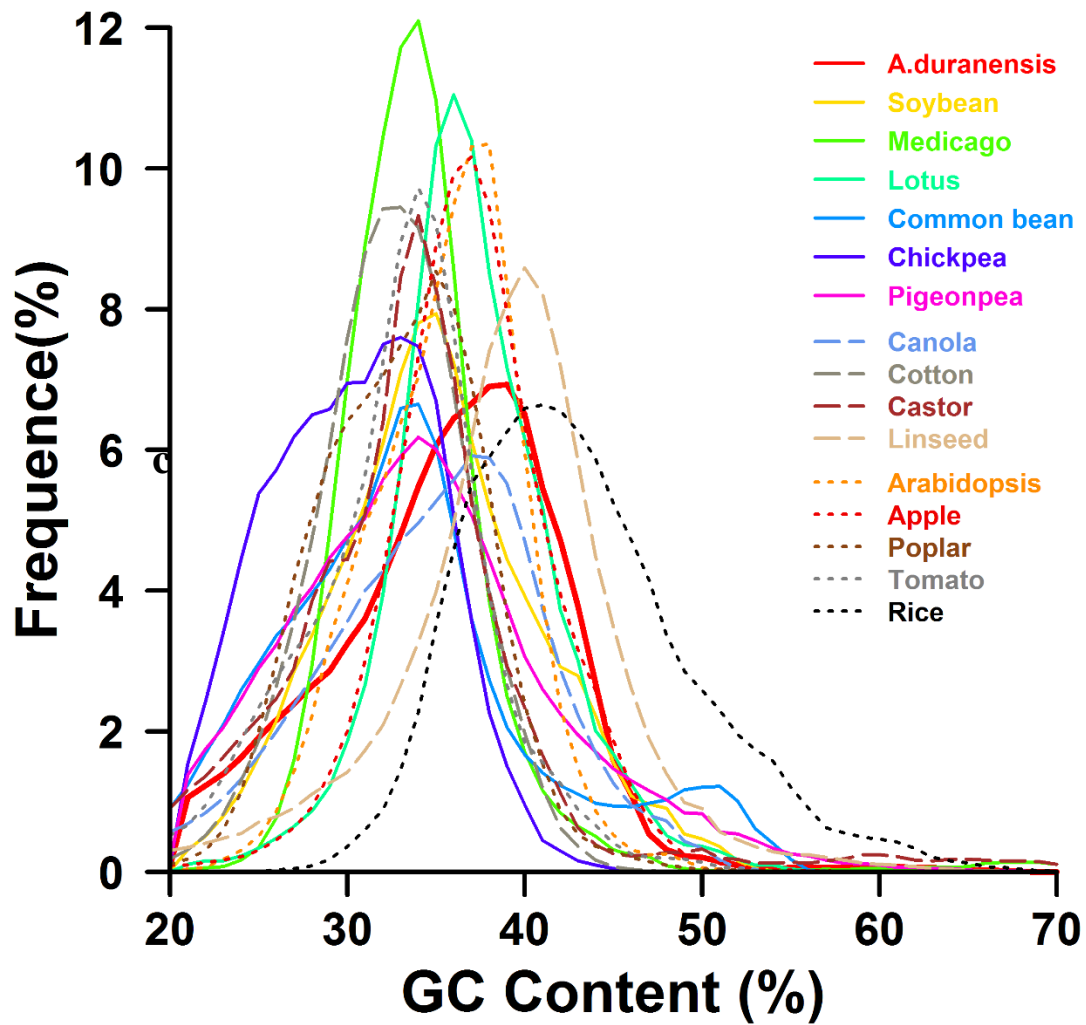


**Figure S6. Coverage of transcripts in the *A. duranensis* de novo assembly.**

The predicted genes were covered by transcripts with  $> 98\%$  identity, and the genes in each coverage were counted ranging from 10% to 100%.

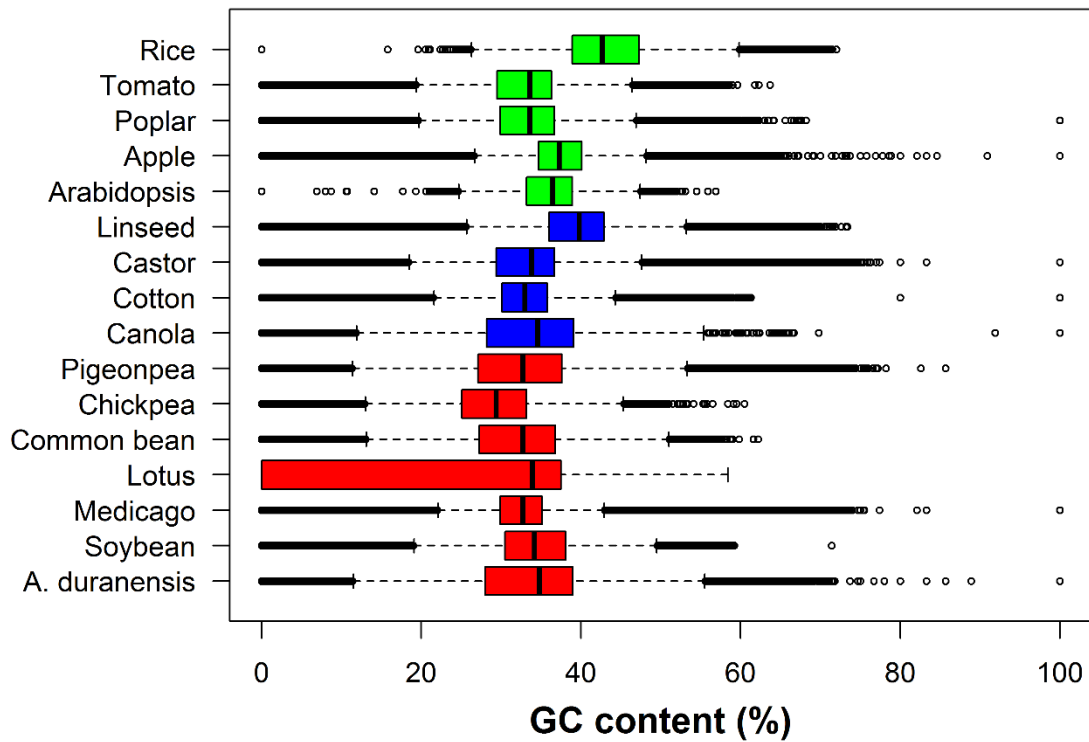


**Figure S7. Boxplot of the heterozygosity in 1-kb window of *A. duranensis* genome.**  
**Heterozygosity in each of 1 kb window was computed and plotted.**  
 The computed heterozygosity matches well with that by AllpathLG (~3 SNPs per kb).



**Figure S8. Comparison of GC content distribution and variation among *A. duranensis*, legumes, oilseeds and other plant species.**  
 Solid lines represent legume species, dash lines represent oilseed species, and dot lines represent other distantly related plant species





**Figure S9. Comparison of the range of GC content among *A. duranensis* and other plant species.**

The boxes display the likely range of the GC content variation (the interquartile range or IQR). The upper and lower bars represent upper and lower inner fences, respectively. The circles depict outliers in the GC content.

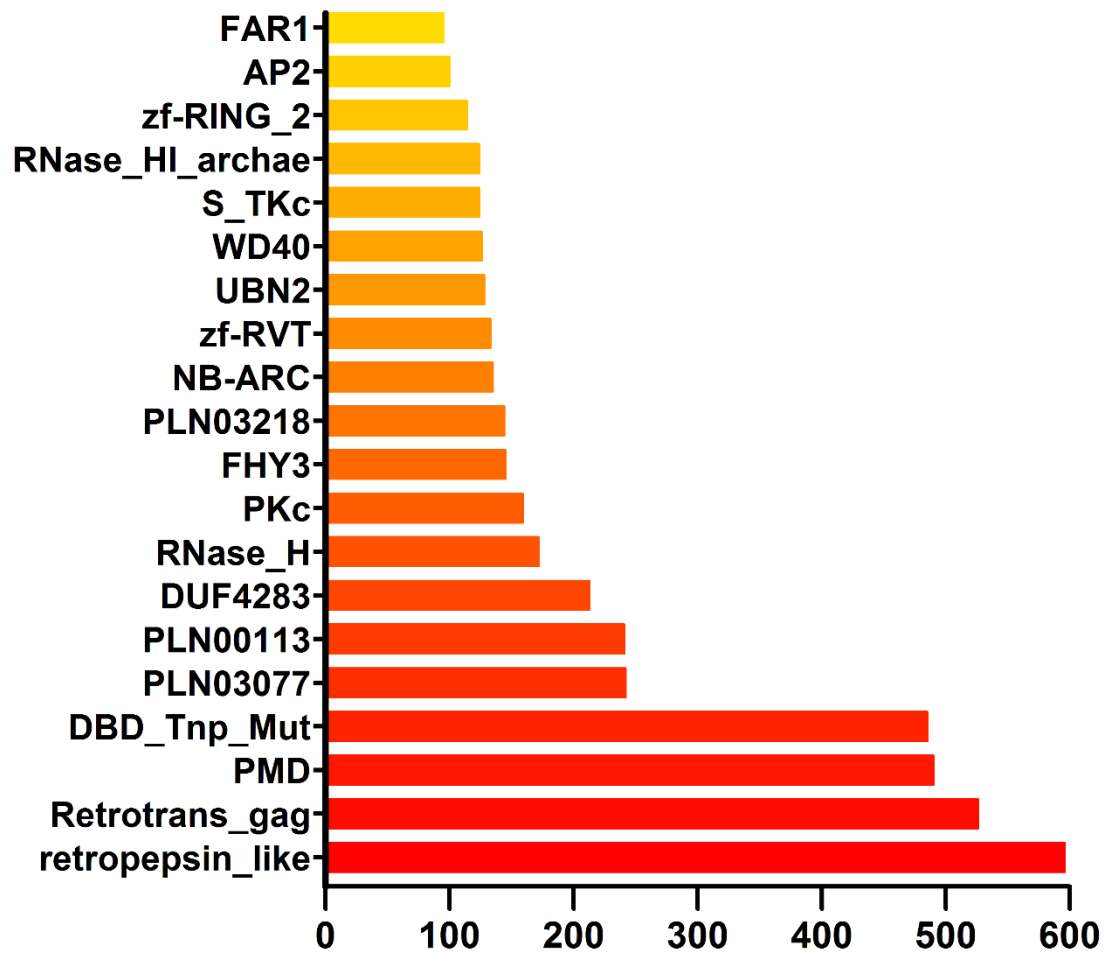
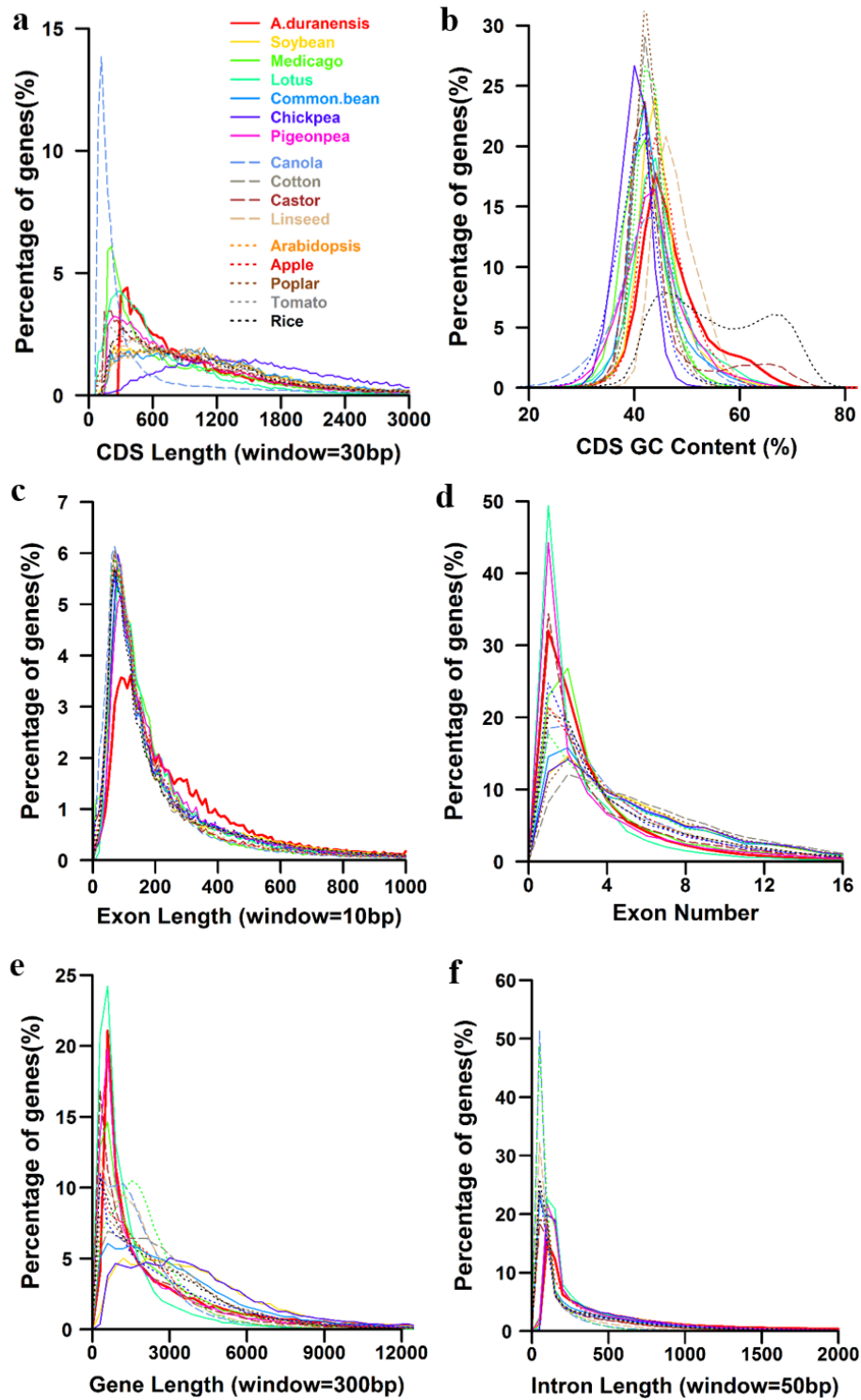


Figure S10. The top 20 Pfam domains for the *A. duranensis* genome.



**Figure S11. Distribution comparison of (a) CDS length, (b) CDs GC content, (c) exon length, (d) exon number, (e) gene length and (f) intron length among *A. duranensis* and other plant species.**

The red solid line presents the distribution in *A. duranensis*. Solid lines represent legume species, dash lines represent oilseed crops and dot lines represent other plant species.

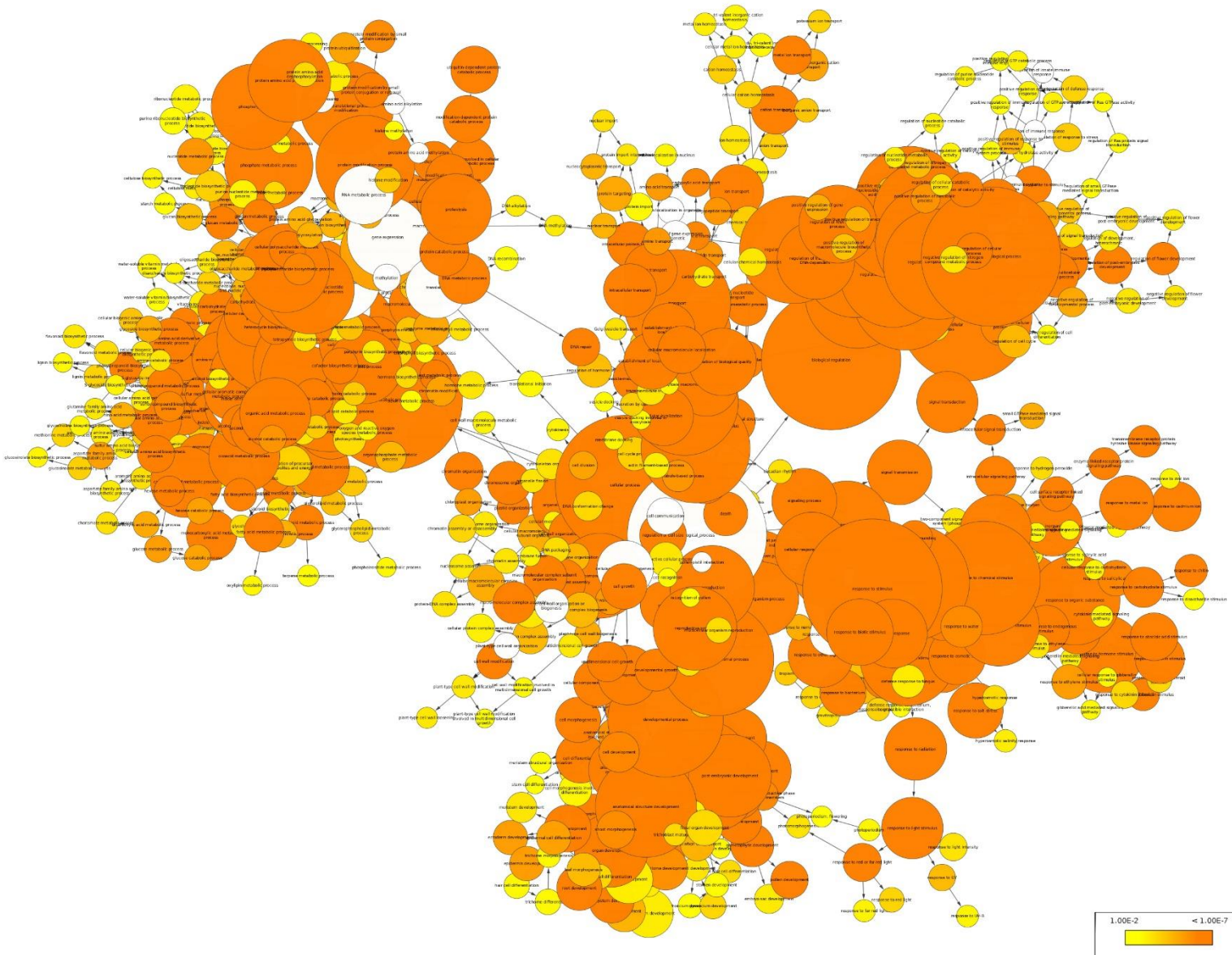
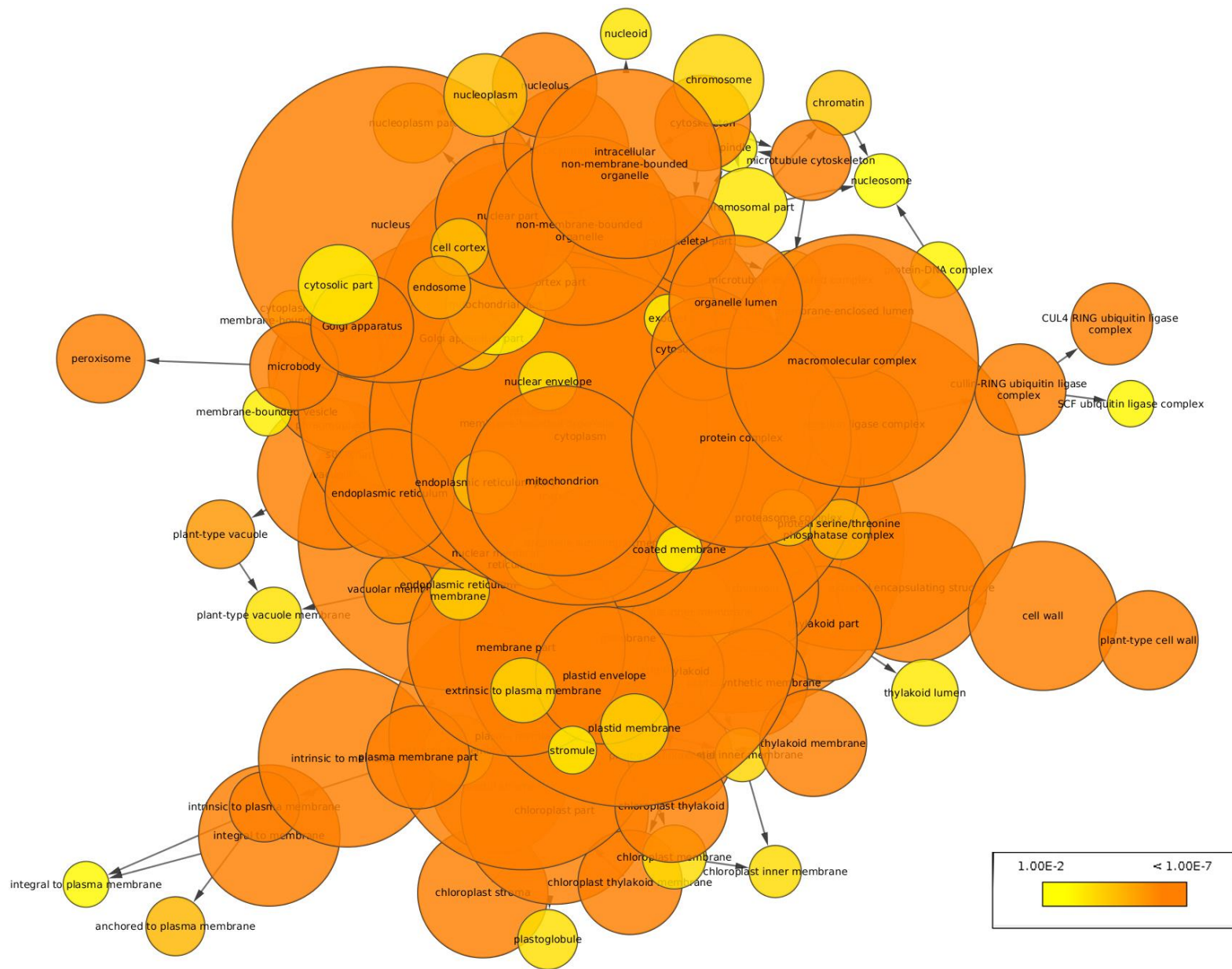


Figure S12. Enriched GO terms for biological process





**Figure S14. Enriched GO terms for cellular components**

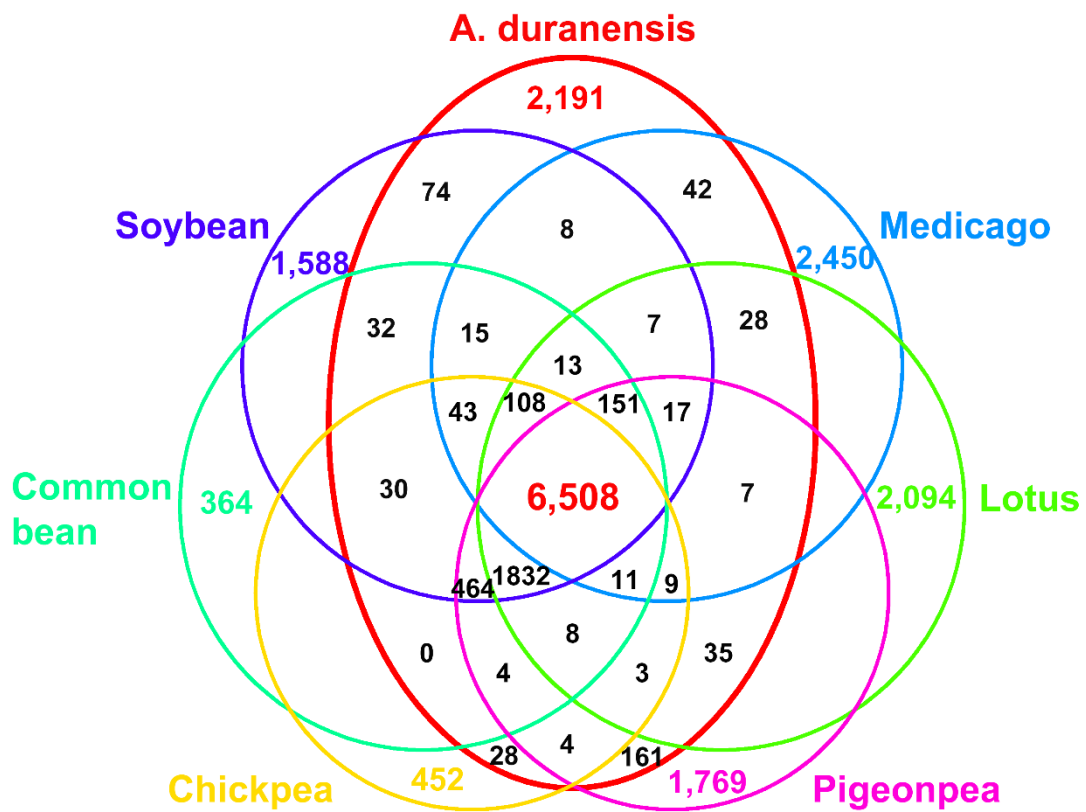
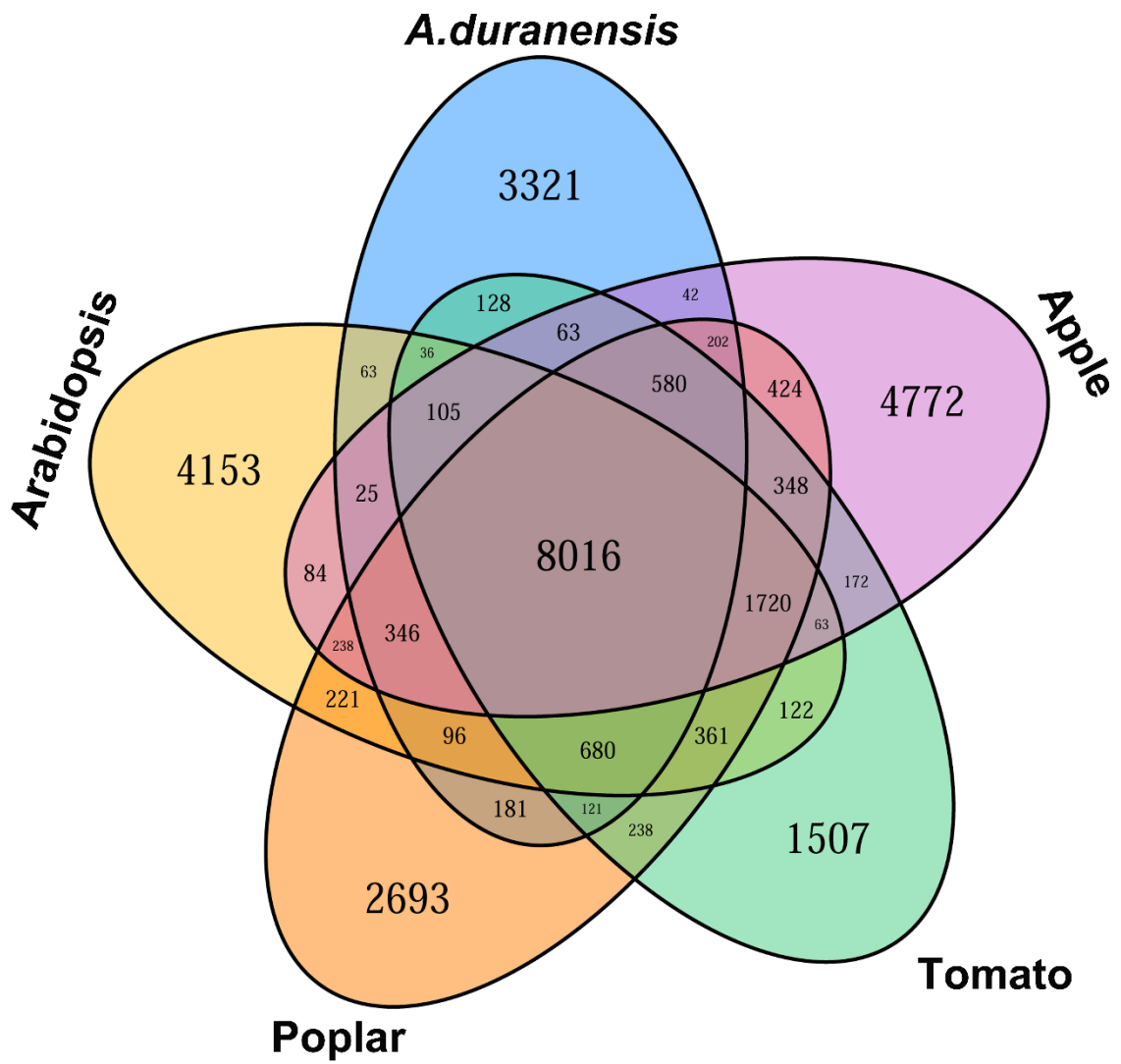


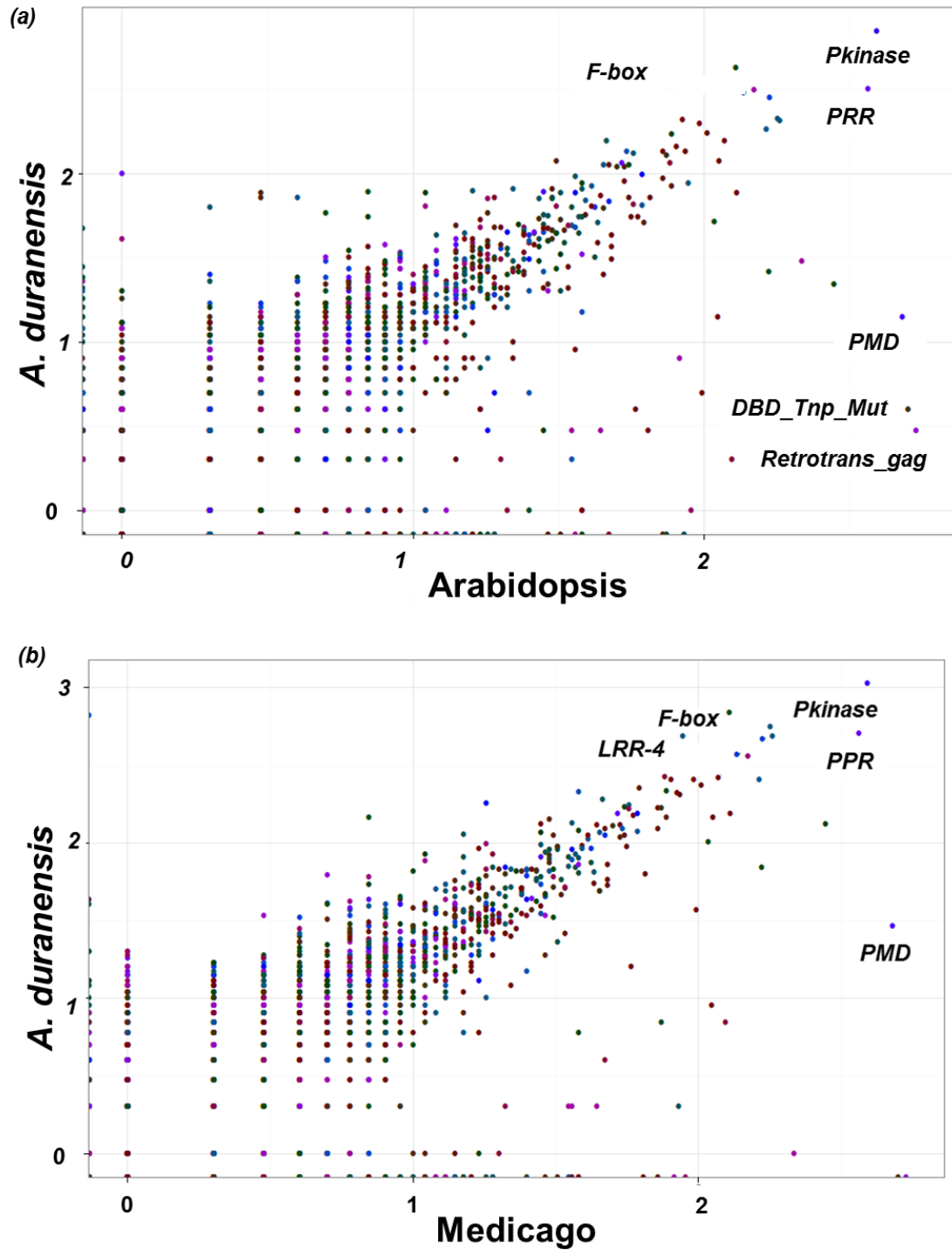
Figure S15. Venn diagram showing shared and unique gene families among legume crops.





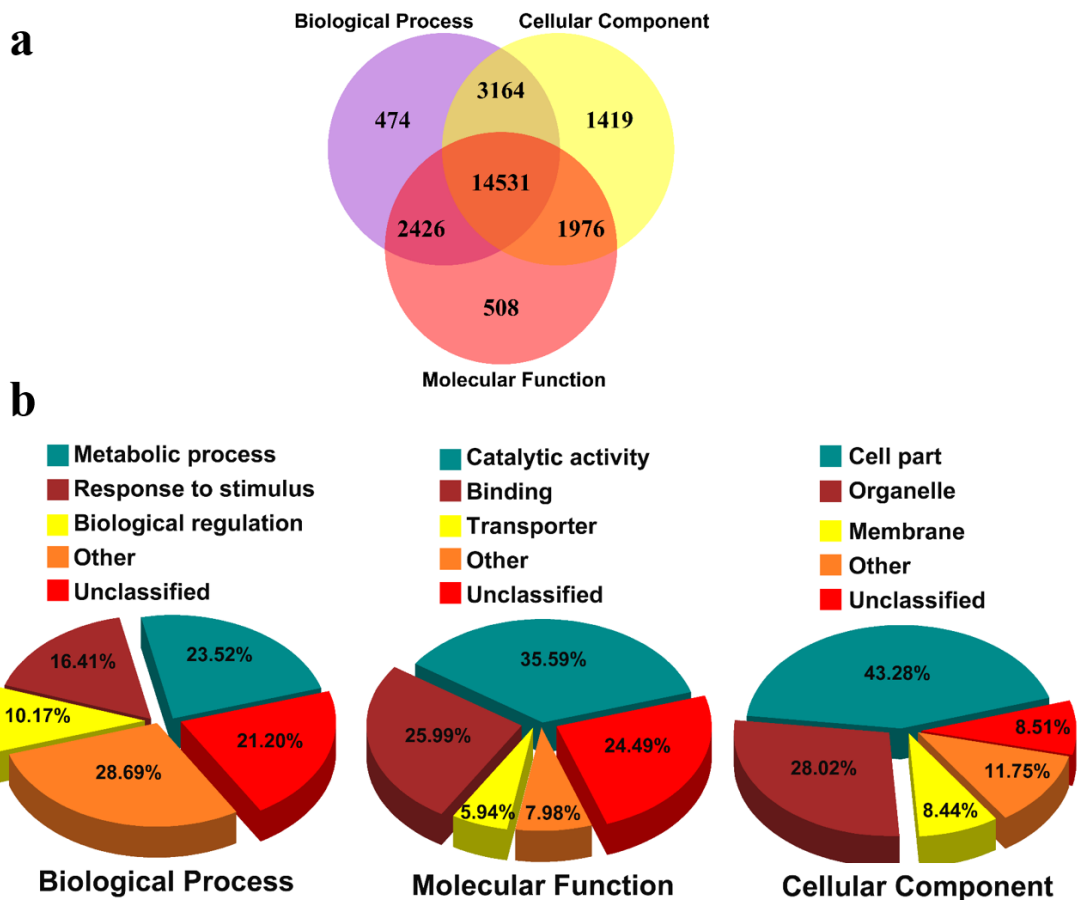


**Figure S17. Venn diagram showing shared and unique gene families among distantly related plant species.**



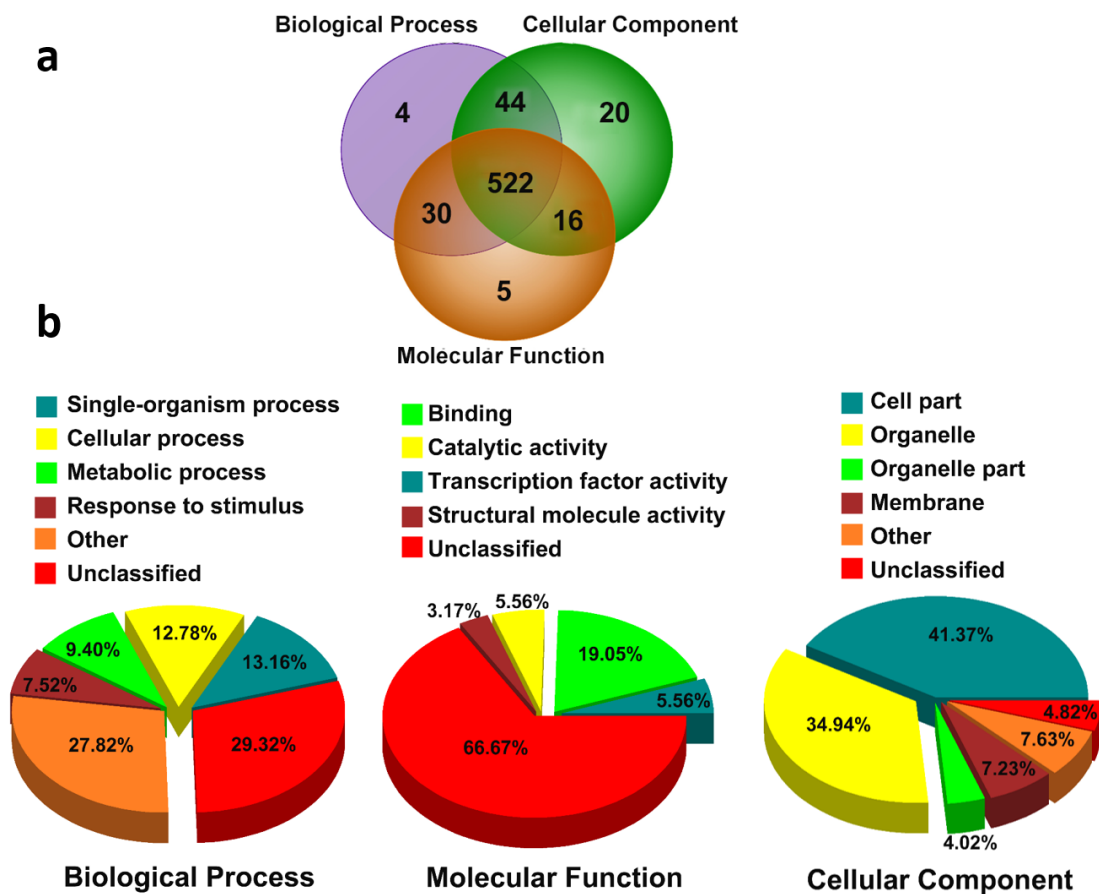
**Figure S18. Pairwise scatterplot of gene family members between *A. duranensis* and *Arabidopsis* as well as *Medicago*.**

The number of members in each family are log10 transformed, and then plotted pairwise. The values >2.5 are only labelled to ease visualization.



**Figure S19. Venn diagram of GO annotation (overlapping genes among three ontologies) in *A. duranensis* predicted protein-coding genes.**

This figure shows (a) the intersection and relationship of each ontology, and (b) the fractions for the top 4 categories in each ontology and the remaining categories.



**Figure S20. Venn diagram of GO annotation (overlapping genes among three ontologies) in *A. duranensis* specific genes.**

This figure shows (a) the intersection and relationship of each ontology, and (b) the fractions for the top 4 or 5 categories in each ontology and the remaining categories.

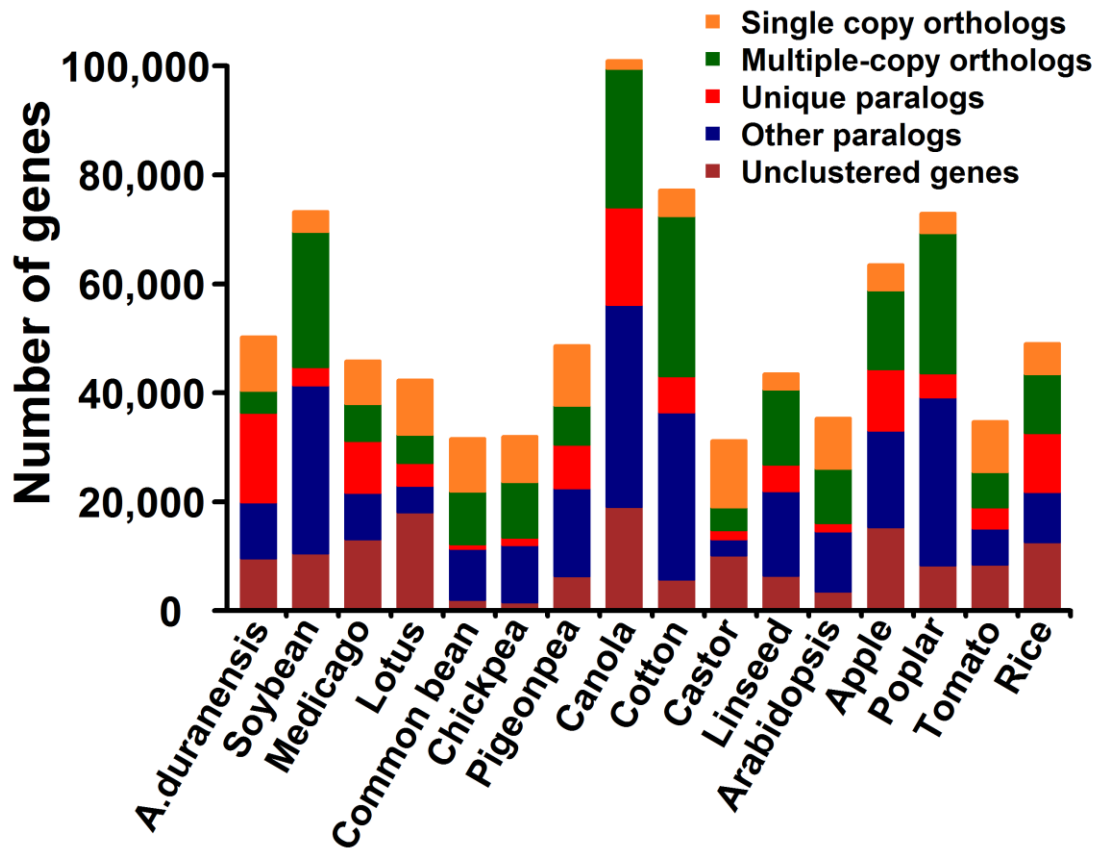


Figure S21. Comparison of orthologous genes among *A. duranensis* and other plant species.

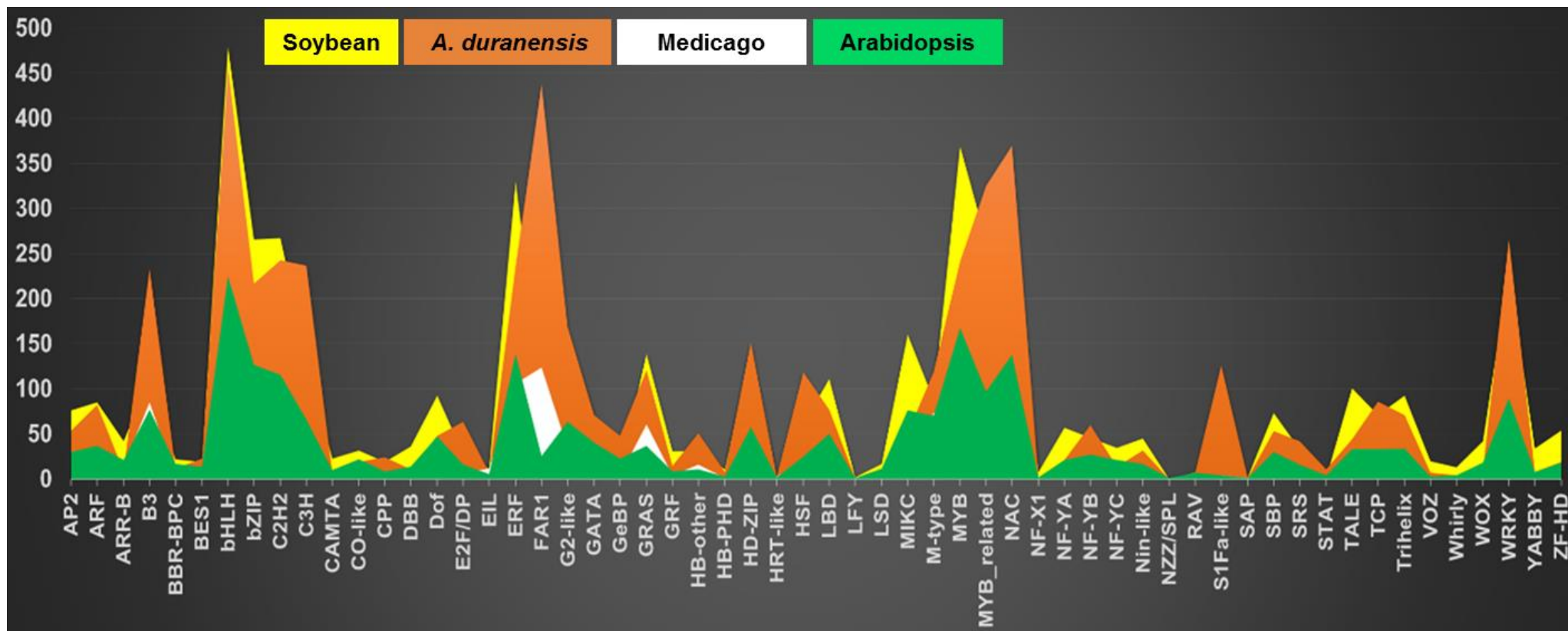
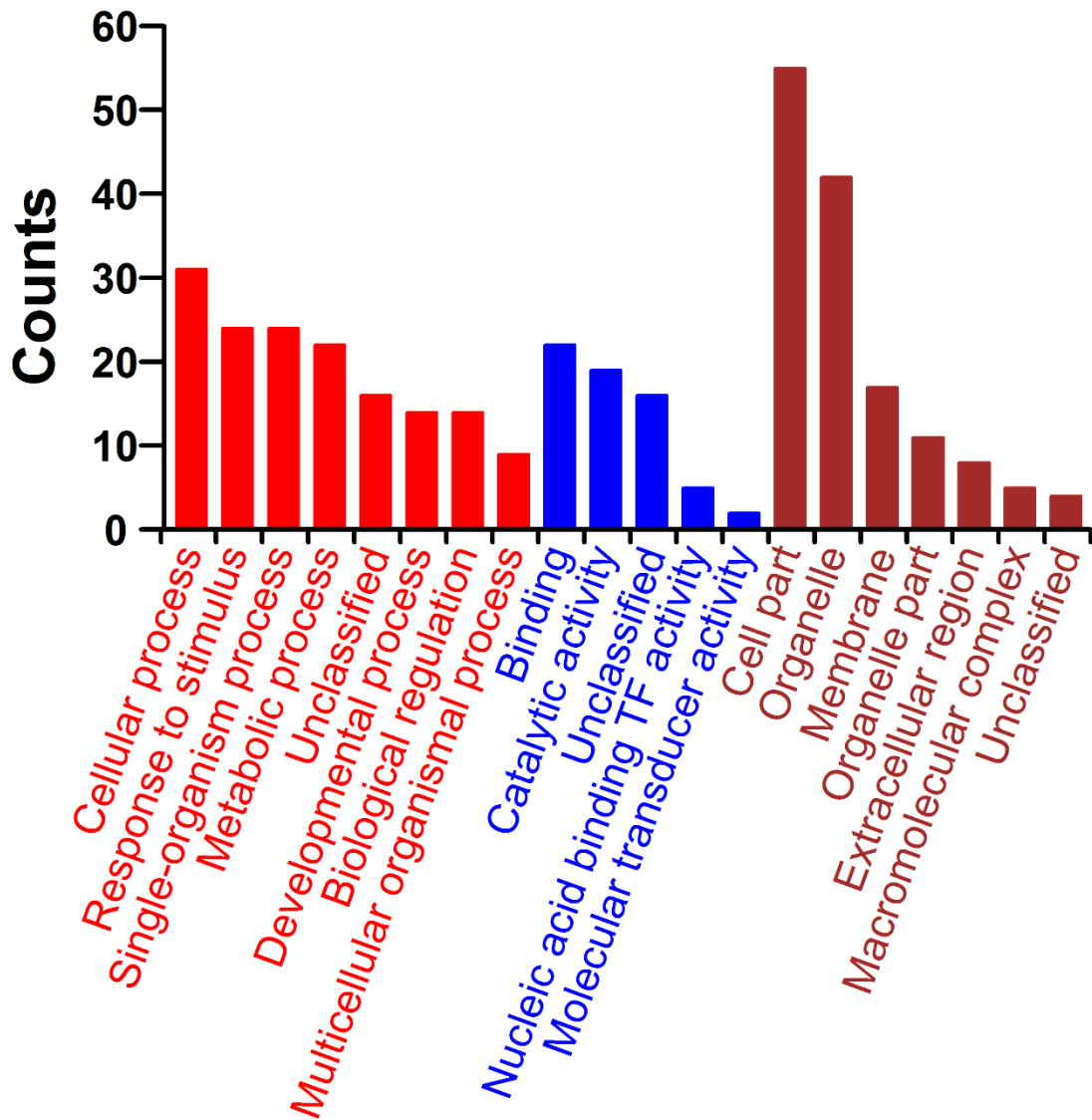


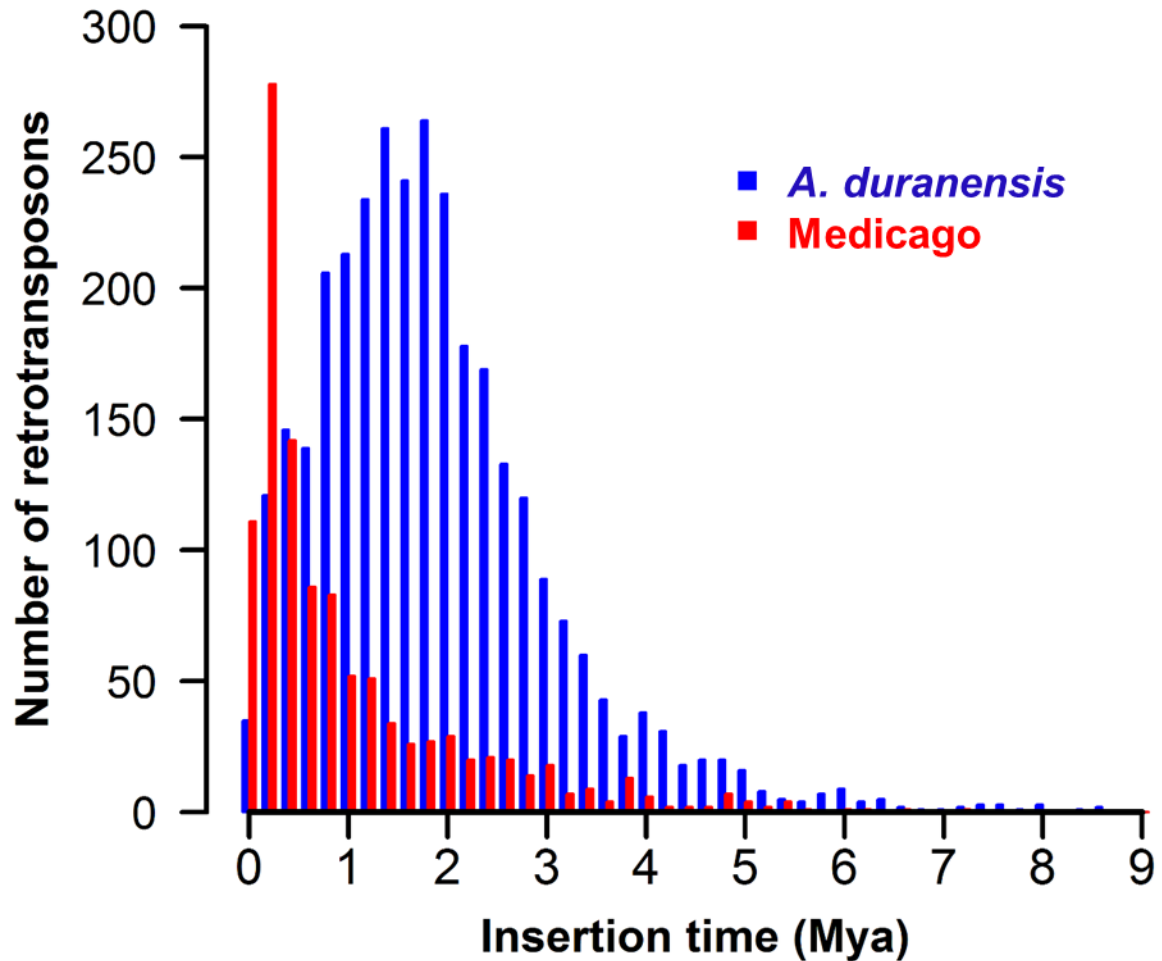
Figure S22. Distribution of TF genes in different TF families among the four species.



**Figure S23. GO classification of miRNA target genes in *A. duranensis*.**

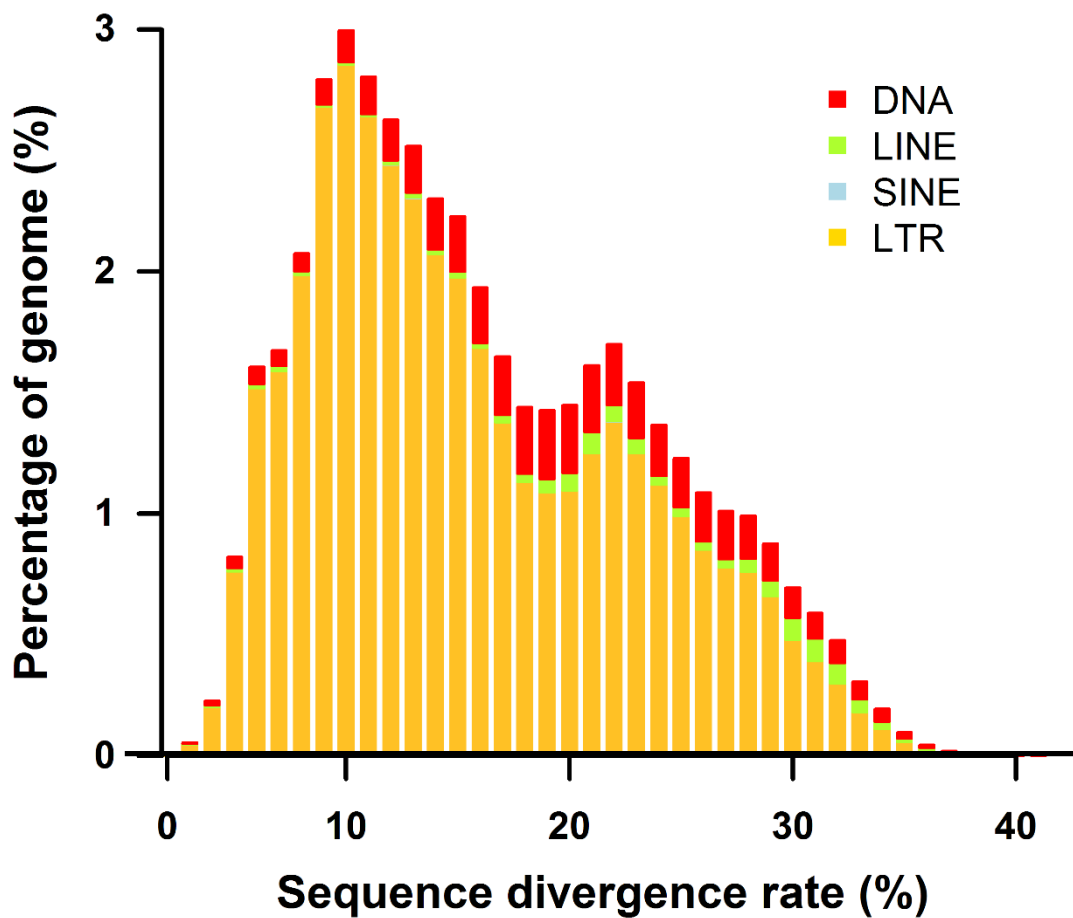
Red colors represent categories of Cellular Component, blue colors represent categories of Biological Process, and brown colors represent categories of Molecular Function.





**Figure S24. Dating the LTR retrotransposon insertion time. Dating of *M. truncatula* LTR retrotransposons was used as a comparison.**

LTR retrotransposon sequences found by LTR\_finder were clustered by CD-HIT at 90% of sequence similarity with 90% coverage of the shorter sequence. The LTR sequences were not included in the calculation of sequence similarity and coverage. The longest in each cluster was selected as the representative member, of which LTRs were aligned and transitions and transversions were computed and used for the insertion time computation.



**Figure S25. Distribution of divergence rate of different repetitive elements (DNA elements, LINE, LTR, SINE) in the *A. duranensis* genome.**

Divergence rate was calculated between the identified TEs in the genome and the consensus sequence in the TE library (Repbases: <http://www.girinst.org/repbases>). DNA, DNA elements; LINE, long interspersed nuclear elements; LTR, long terminal repeat transposable element; SINE, short interspersed nuclear elements.

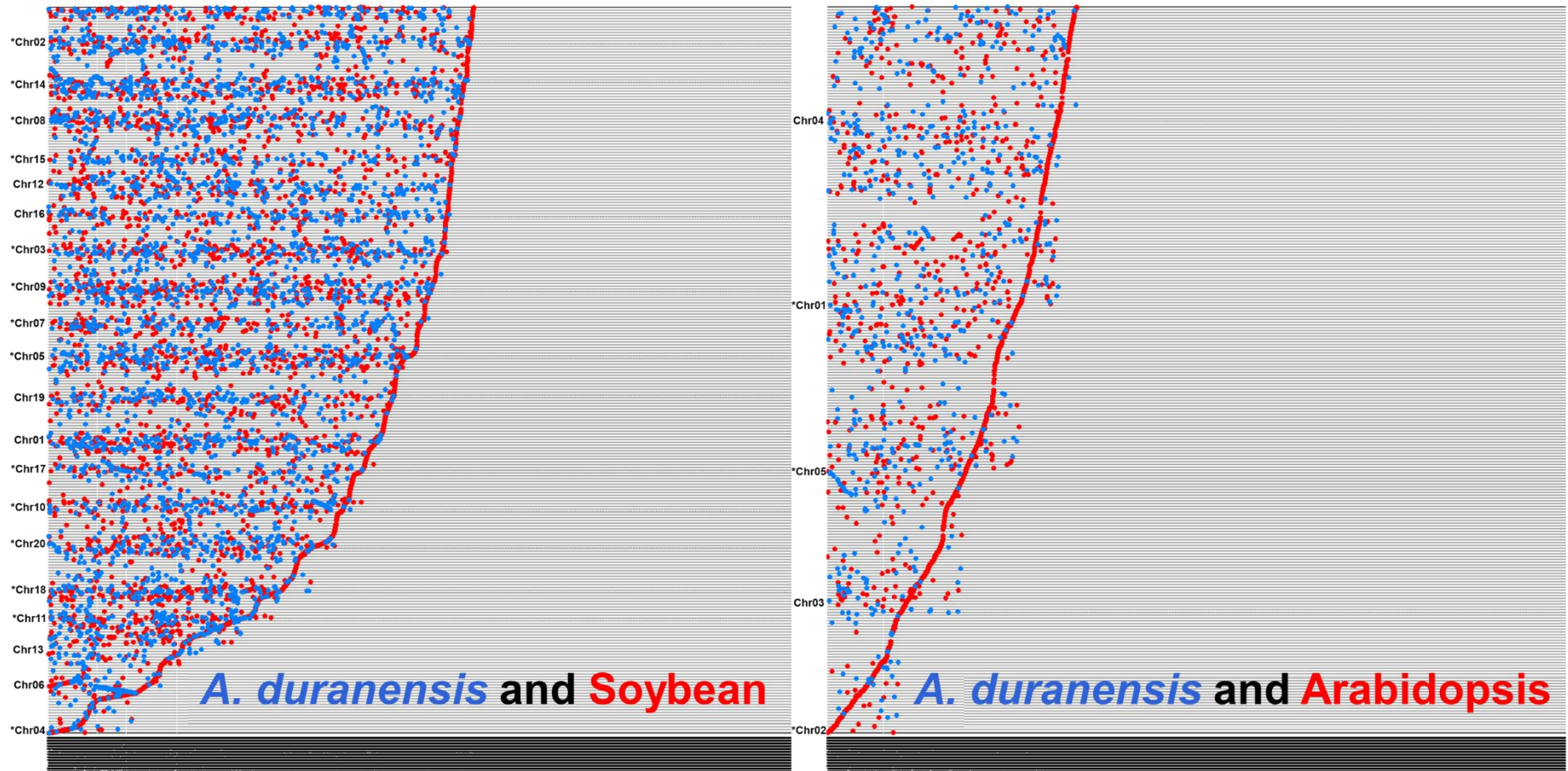


Figure S26. Syntenic blocks between *A. duranensis* scaffolds and Soybean and Arabidopsis chromosomes.

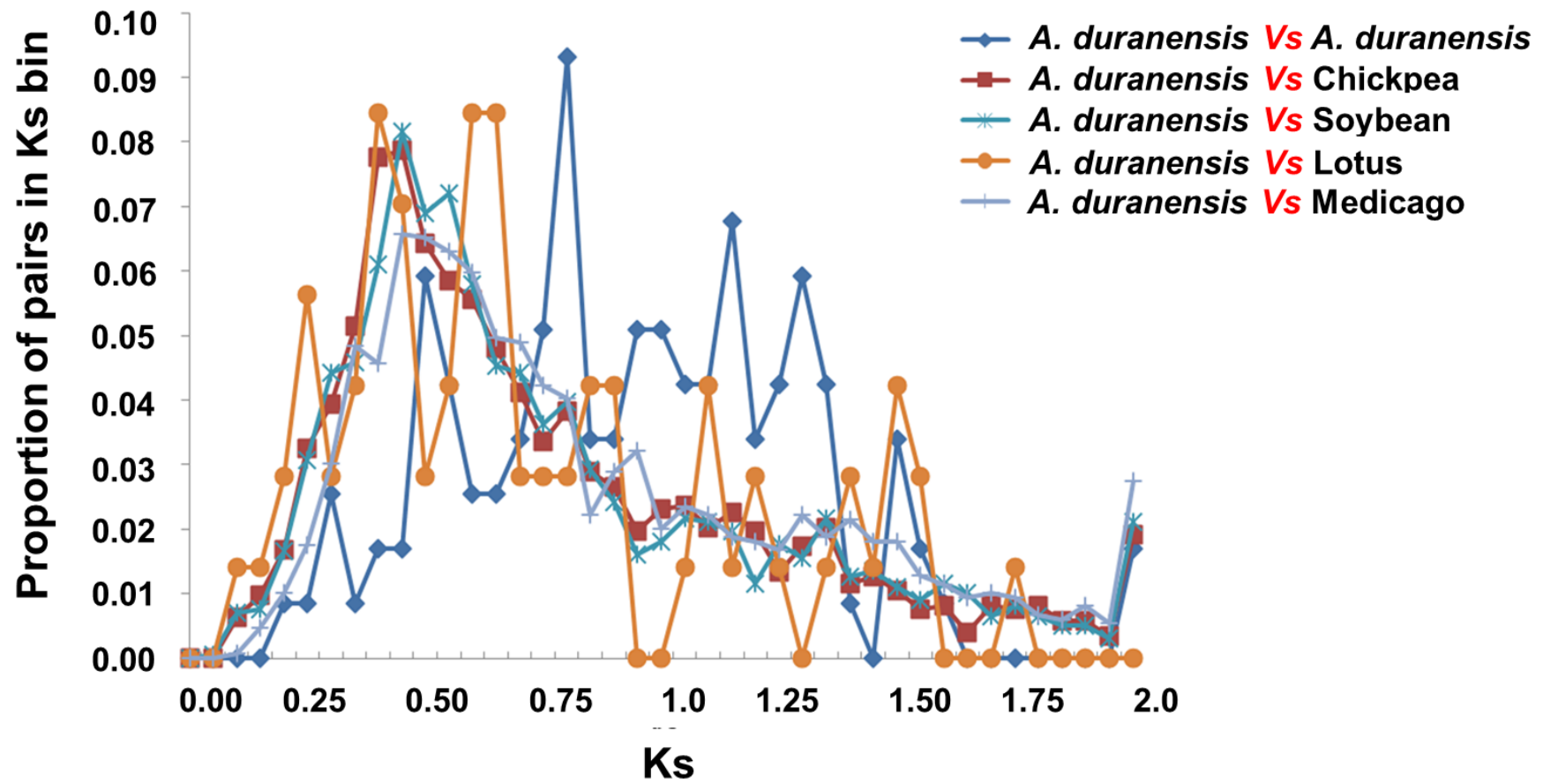


Figure S27. Ks analysis of legume species.

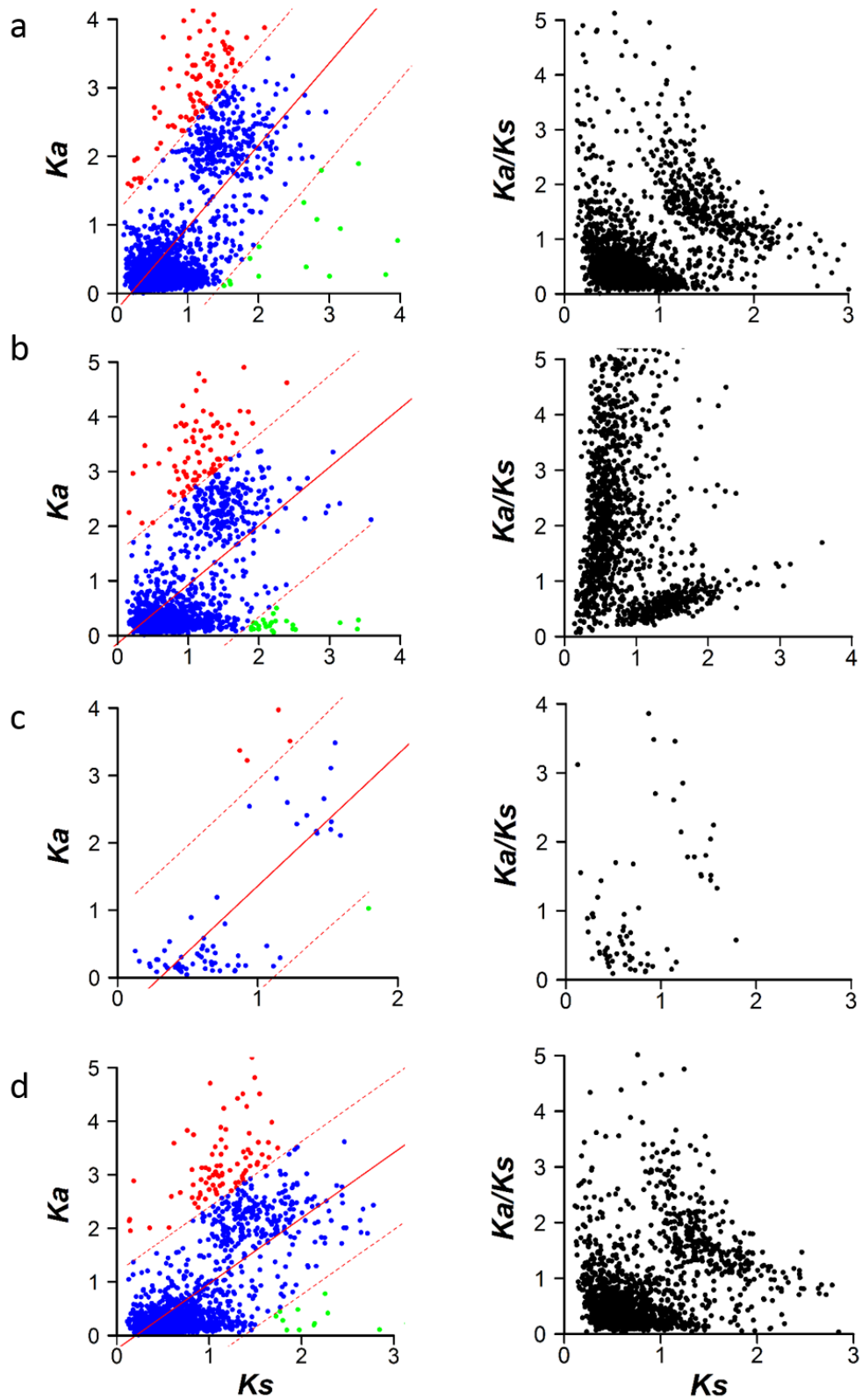
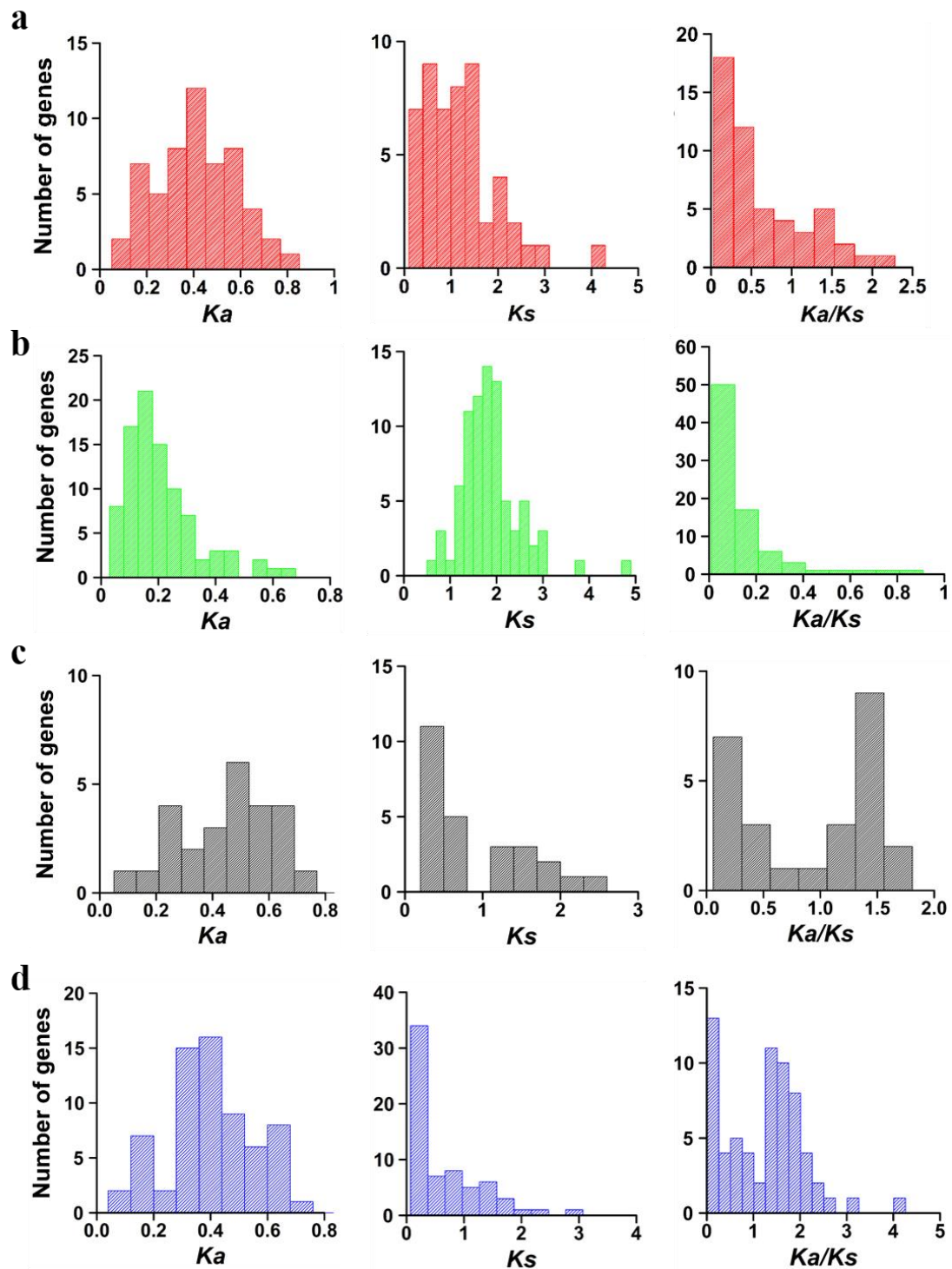


Figure S28. Scatterplot of  $K_s$  vs.  $K_a$  of orthologs between *A. duranensis* and soybean (a), *Medicago* (b), *Lotus* (c) and pigeonpea (d).

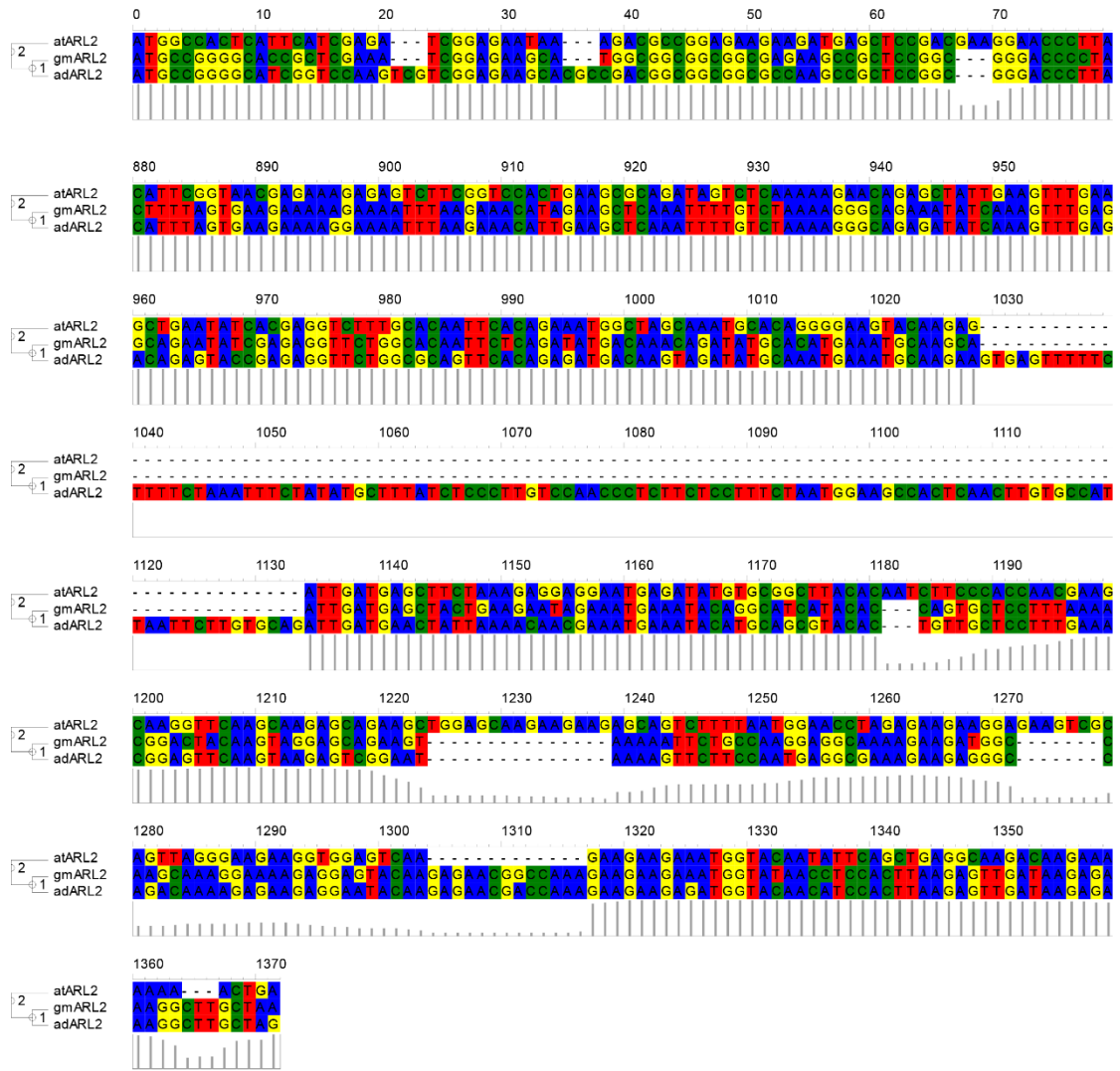
The dashed line represents the prediction interval about the linear regression. Red and green dots represent high and low  $\omega$  ( $Ka/Ks$ ) gene pairs, respectively.



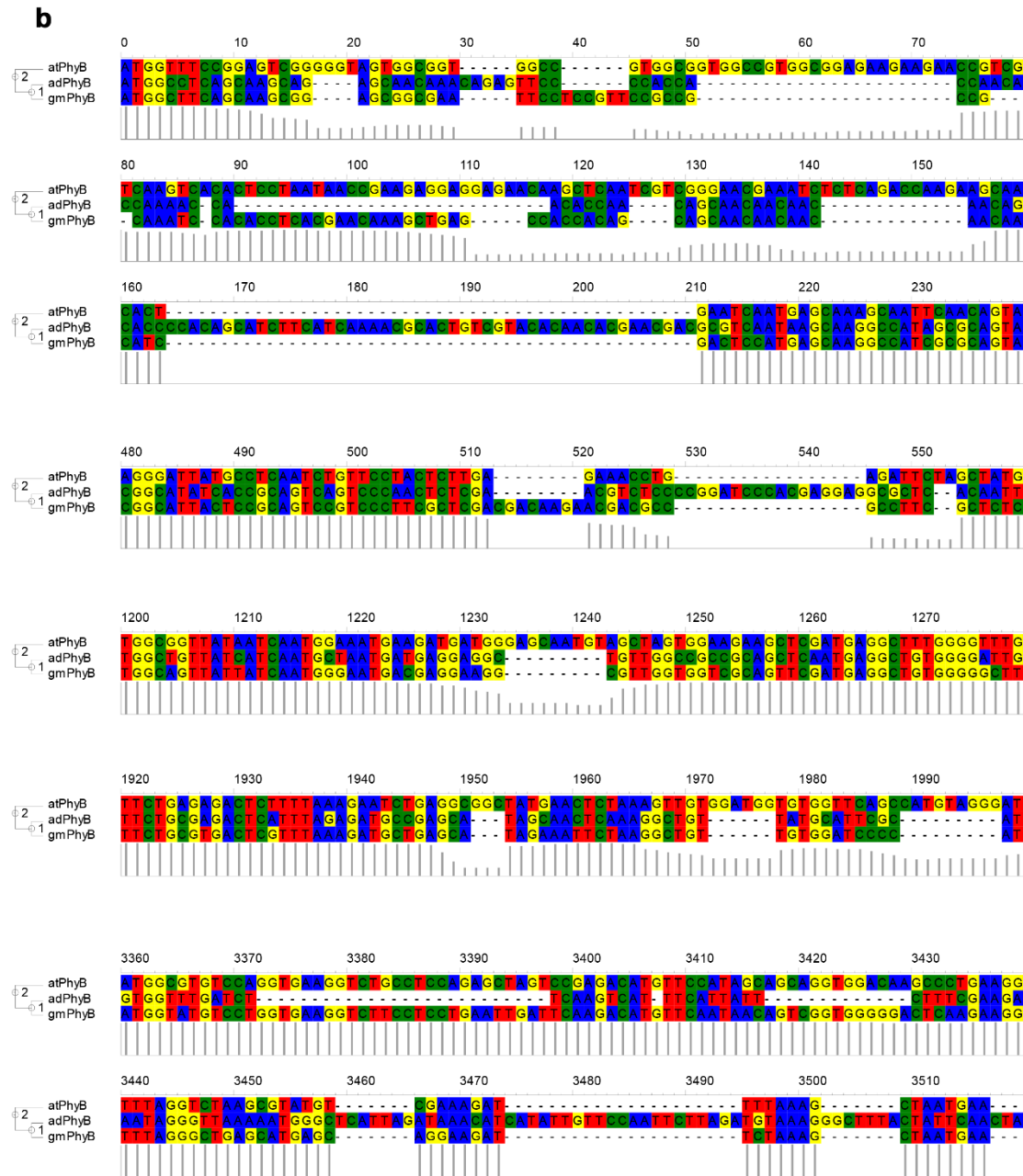
**Figure S29.** Distribution of  $Ka$ ,  $Ks$  and  $\omega$  ( $Ka/Ks$ ) in pairs of (a) *Arabidopsis* and *A. duranensis* genes involved in gravitropism as well as in (b) *Arabidopsis* and

soybean. (c) Distribution of  $Ka$ ,  $Ks$  and  $\omega$  in pairs of *Arabidopsis* and *A. duranensis*  
(c) genes related to photomorphogenesis as well as of (d) *Arabidopsis* and soybean.

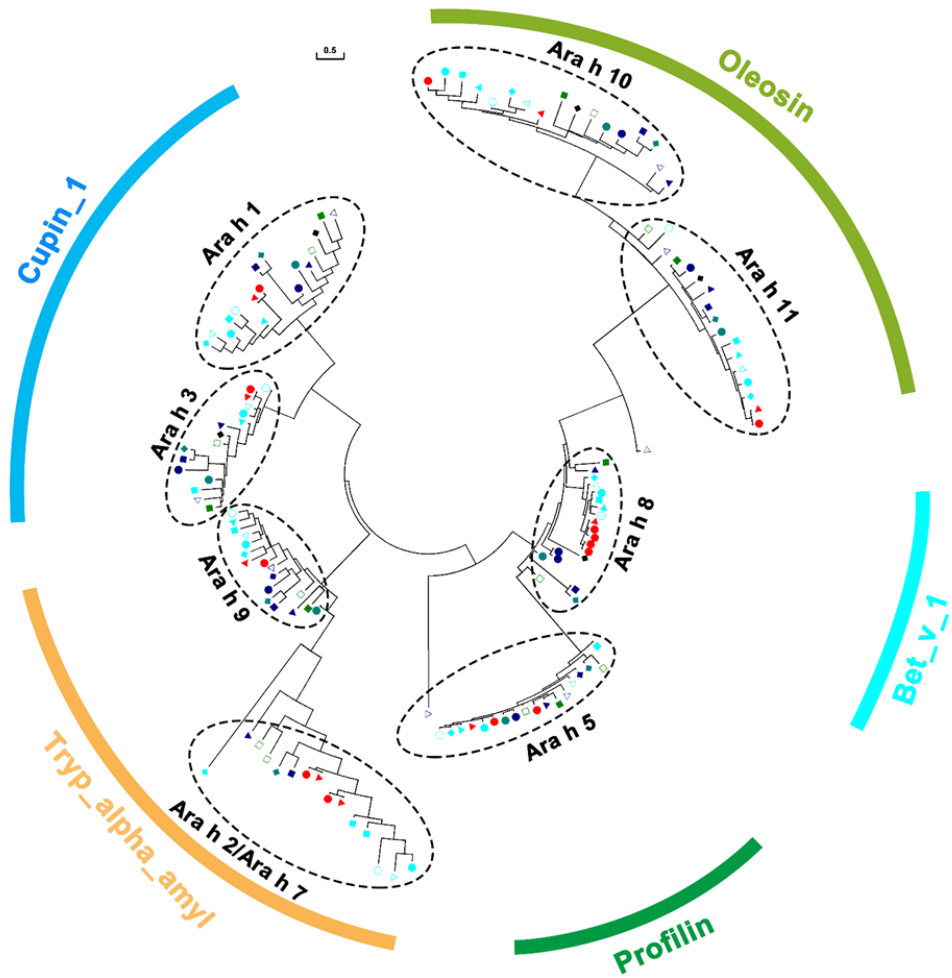
**a**





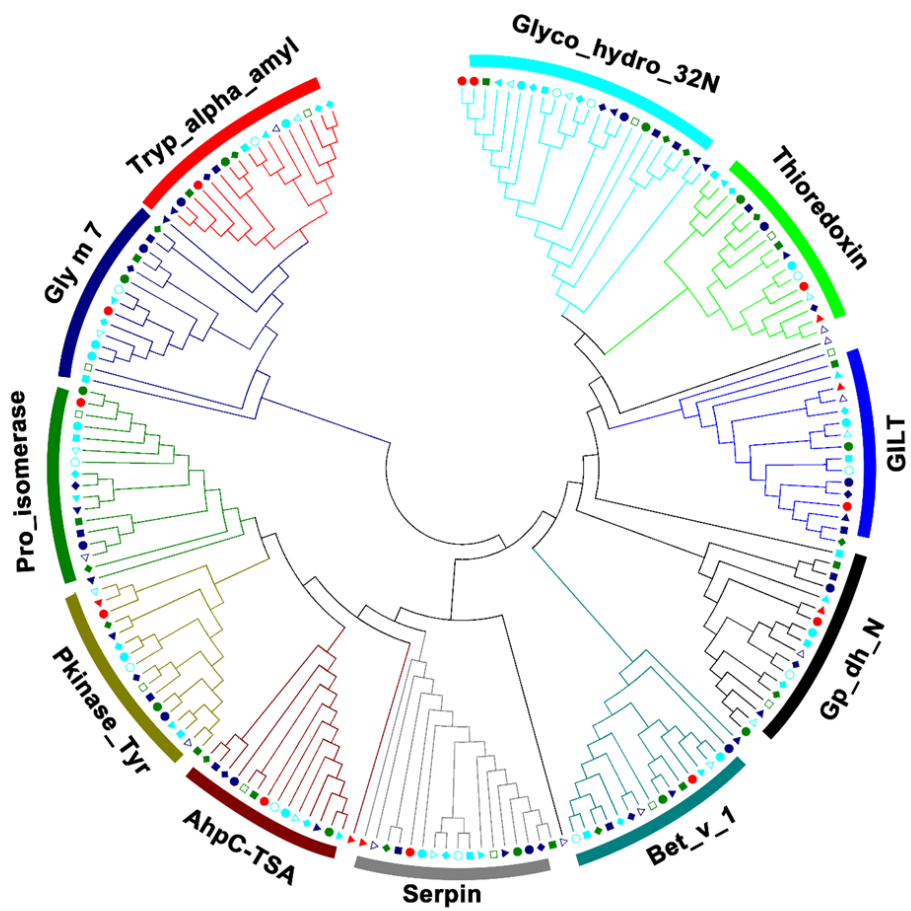


**Figure S30. SNPs and Indels of representative genes under positive selection for gravitropism *ARL2* (a) and photomorphogenesis *phyB* (b) in *A. duranensis* (ad), *Arabidopsis* (at) and soybean (gm).** Phylogeny-aware alignments of these genes were performed using PRANK and visualized using PRANKSTER. The approximate guide trees is indicated in left for each alignment. Alignments resulting in large-effect indel are shown.



- *A. duranensis*    ▲ *A. hypogaea*    ● Soybean    ■ Medicago    ▲ Lotus    ◆ Common bean
- Chickpea    △ Pigeonpea    ◆ Canola    ● Cotton    □ Castor    ■ Linseed
- Arabidopsis    ● Apple    ◆ Poplar    ▲ Tomato    △ Rice

**Figure S31. Phylogenetic tree of *Ara h 1-11* allergens, including sequences from previously identified homologs from cultivated peanut and other species.**



- |                        |             |           |            |          |               |
|------------------------|-------------|-----------|------------|----------|---------------|
| ● <i>A. duranensis</i> | ▲ wheat     | ● Soybean | ■ Medicago | ▲ Lotus  | ◆ Common bean |
| ○ Chickpea             | △ Pigeonpea | ◆ Canola  | ● Cotton   | □ Castor | ■ Linseed     |
| ■ Arabidopsis          | ● Apple     | ◆ Poplar  | ▲ Tomato   | △ Rice   |               |

**Figure S32. Phylogenetic tree of newly identified putative allergens in *A. duranensis* and the homologous proteins in other plant species.**

## SI References

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