Strategies of tolerance reflected in two North American maple genomes

Susan L. McEvoy¹, U. Uzay Sezen², Alexander Trouern-Trend¹, Sean M. McMahon², Paul G. Schaberg³, Jie Yang⁴, Jill L. Wegrzyn¹, Nathan G. Swenson⁵

¹ Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut, 06269

² Smithsonian Environmental Research Center, Edgewater, Maryland, 21037

³ Forest Service, U.S. Department of Agriculture, Northern Research Station, Burlington, VT 05405

⁴ CAS Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, 666303, Yunnan, China

⁵ Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, 46556

#Corresponding authors: Jill Wegrzyn, email: jill.wegrzyn@uconn.edu, Nate Swenson, email nswenson@nd.edu

18 **Abstract**

19 Maples (the genus *Acer*) represent important and beloved forest, urban, and ornamental trees
 20 distributed throughout the Northern hemisphere. They exist in a diverse array of native ranges and
 21 distributions, across spectrums of tolerance or decline, and have varying levels of susceptibility to
 22 biotic and abiotic stress. Among *Acer* species, several stand out in their importance to economic
 23 interest. Here we report the first two chromosome-scale genomes for North American species,
 24 *Acer negundo* and *Acer saccharum*. Both assembled genomes contain scaffolds corresponding to
 25 13 chromosomes, with *A. negundo* at a length of 442 Mb, N50 of 32 Mb and 30,491 genes, and *A.*
 26 *saccharum* at 626 Mb, N50 of 46 Mb, and 40,074 genes. No recent whole genome duplications
 27 were detected, though *A. saccharum* has local gene duplication and more recent bursts of
 28 transposable elements, as well as a large-scale translocation between two chromosomes. Genomic
 29 comparison revealed that *A. negundo* has a smaller genome with recent gene family evolution that
 30 is predominantly contracted and expansions that are potentially related to invasive tendencies and
 31 tolerance to abiotic stress. Examination of expression from RNA-Seq obtained from *A.*
 32 *saccharum* grown in long-term aluminum and calcium soil treatments at the Hubbard Brook
 33 Experimental Forest, provided insights into genes involved in aluminum stress response at the
 34 systemic level, as well as signs of compromised processes upon calcium deficiency, a condition
 35 contributing to maple decline.

36

37 **Introduction**

38 *Acer saccharum* (sugar maple) is a long-lived and dominant species in New England forests with
 39 a native range representing Eastern Canada, and the Northcentral and Northeastern United States.
 40 Widely known for its vibrant autumn hues, high quality timber, and as the preferred species for
 41 the production of maple syrup, *A. saccharum* also plays a key role in its native ecosystems,
 42 altering soil mineral content (Lucash et al., 2012), moisture levels (Emerman & Dawson, 1996),
 43 and mycorrhizae communities (Cong et al., 2015). *A. saccharum* provides food and shelter to
 44 many mammals (Godman et al., 1990), resident and migratory birds (Flashpohler and
 45 Grosshuesch 1996; Weidensaul et al. 2020), and over 300 species of caterpillar. Although seeds

46 from this predominantly monoecious species are wind dispersed, the early flowers are an
47 important pollen source for bees in late winter (Blitzer et al., 2016).

48

49 One of the most phylogenetically (~50 MY) and morphologically distinctive *Acer* species from *A.*
50 *saccharum* is *Acer negundo* (box elder). *A. negundo* is a short-lived tree that has the largest range
51 of all North American *Acer*. It is native to predominantly lower elevation regions of Canada, the
52 United States, and Mexico ([Figure 1](#); Enquist et al., 2016; Maitner et al., 2018). This adaptable
53 pioneer species is often seen in disturbed sites and urban settings. *A. negundo* has soft wood, less
54 concentrated sugars for syrup, grows rapidly, and can tolerate low nutrient soils, moderate
55 salinity, and drought conditions. Its invasive status in large portions of Europe, South America,
56 and Asia are indicative of greater phenotypic plasticity (Lamarque et al., 2013). *A. negundo* has a
57 number of distinctive attributes including dioecy (Renner et al., 2007) and pinnately compound
58 leaves, mostly seen only in close relatives. Within such a significant genus, these two species
59 reflect a breadth of social and ecological diversity and importance that recommends better
60 understanding of their genetic distinctions.

61

62 Forest ecosystems of the Northeastern U.S. are facing significant changes in composition driven
63 by climate change (Rogers et al., 2017). “Maple decline” is a term referring to the loss of maple
64 populations, originally referring to *A. saccharum*, but now applicable to *A. platanoides* and *A.*
65 *rubrum* in the Northeast, and most recently, *A. macrophyllum* in the Northwest. Loss of *A.*
66 *saccharum* has been documented over the last century, beginning in the late 1950s, leading to the
67 first comprehensive, multidisciplinary study of this condition (Giese & Benjamin, 1964; Horsley
68 et al., 2002). Maple decline is characterized by crown dieback, reduction in overall health and
69 vigor, and a decrease in regeneration (Bishop et al., 2015). Episodic decline has increased in
70 recent decades (Oswald et al., 2018). Decline and crown dieback of dominant *A. saccharum*
71 provides a release for sympatric species such as *Fagus grandifolia* (American beech) which
72 displays a higher level of tolerance to soil conditions and foliar aluminum ratios leading to
73 shifting forest composition (Halman et al., 2015). Studies examining potential factors of maple
74 decline have largely agreed that modified soil conditions, largely due to acid deposition, are the

75 leading cause, compounded by additional climatic, pathogenic, and anthropogenic stressors (Bal
76 et al., 2015). Acidic soils rapidly leach the essential cations calcium, magnesium and potassium,
77 while mobilizing aluminum within the soil and contributing to more phytotoxic forms (Likens et
78 al., 1998; Likens & Lambert, 1998). Competition between aluminum and calcium at the roots
79 further decreases levels of available calcium within tissues, while increasing aluminum damages
80 plasma membranes, cell walls, DNA, and increases the burden of oxidative stress. Such nutrient
81 interactions and their broader consequences on physiology and ecology are studied at the Hubbard
82 Brook Experimental Forest (HBEF), a Long Term Ecological Research (LTER) site. It was here
83 that acid deposition was first discovered in North America (Likens and Bormann 1974) and
84 continues to be studied through the Nutrient Perturbation (NuPert) program (Berger et al., 2001).
85 It provides a replicated high elevation natural ecosystem to examine current, future, and past soil
86 conditions, and has been the site of several key studies on native species, including *A. saccharum*,
87 *A. balsamea*, *F. grandifolia*, and *P. rubens*. At HBEF, no studies to-date on *A. saccharum* have
88 focused at the genomic level, where variation in gene expression or signs of adaptation among
89 gene families may be more immediately informative in these slow-growing organisms. For such
90 analysis, a high-quality, chromosomal-length genome is necessary to more accurately detect these
91 forms of variation.

92

93 Genomic resources necessary to guide *Acer* conservation are very limited. Only two genomes
94 exist to-date: *A. yangbiense*, native to the Yuhang Province (J. Yang et al., 2019) and *A. truncatum*
95 (purpleblow maple), widely distributed across East Asia (Ma et al., 2020). Here, we present the
96 first two North American *Acer* genomes, *A. saccharum* and *A. negundo*. With these
97 chromosome-scale references, we describe differences in genomic characteristics that may reflect
98 their alternative tolerance strategies. We conducted a differential expression study with stem
99 tissue from *A. saccharum* individuals from HBEF in order to identify genes that may be involved
100 in aluminum response and calcium deficiency. Identification of key processes in the expression
101 study helped to provide focus to the following analysis of comparative gene family dynamics.
102 Together, these approaches highlighted families associated with various abiotic stress responses,
103 including those that also have significant dynamics or novel isoforms in *A. saccharum* or *A.*

104 *negundo* relative to other broadleaf tree species. And it allowed investigation of the effects of
 105 calcium availability at the molecular level, a significant factor associated with maple decline.
 106

108
 109 **Figure 1.** a) Native distributions of *A. saccharum* (blue) and *A. negundo* (orange) in North
 110 America. Leaves indicate location of individuals selected for the reference genomes; *A.*
 111 *saccharum* from the University of Maryland campus, and *A. negundo* from the Smithsonian
 112 Environmental Research Center. HBEF (Hubbard Brook Experimental Forest) is the location of

the 9 individuals used for RNA-seq. b) All records of occurrence, including native, introduced, and unknown, per BIEN 4.2. Non-native occurrences are predominantly *A. negundo*.

Results

Genome size estimation and quality control

A. saccharum DNA sequencing resulted in 63 Gb of PacBio data with an N50 of 21 Kb (max read length 94 Kb) and Illumina PE data totalling 225 Gb. *A. negundo* was similar with 61 Gb of PacBio data, an N50 of 17 Kb (max read length 95 Kb), and 223 Gb of Illumina PE data. Genome size estimation using short reads resulted in smaller than expected estimates (Contreras & Shearer, 2018; Leitch et al., 2019) at 636 Mb and 319 Mb for *A. saccharum* and *A. negundo*, respectively [Figure S1](#). Using the short-read estimations of genome length, DNA sequence read coverage was high, with long reads at 111x and 141x and short reads at 180x and 208x for *A. saccharum* and *A. negundo*, respectively ([Table S1](#)). RNA sequencing of the reference individuals resulted in 61 M reads for *A. saccharum* (92% mapped) and 62 M for *A. negundo* (93% mapped). RNA sequencing of samples for the differential expression study resulted in 207 M reads with mapping rates that ranged from 82 to 92%.

Genome assembly

Testing of multiple assembly approaches found the FALCON/Purge Haplotigs assembly to be the most contiguous and closest to *A. saccharum*'s estimated size ([Figure 2](#)). Statistics for the set of primary contigs from FALCON did not change much between the assembly, unzip, and polishing stages ([File S1](#)). The final total length for *A. saccharum* settled at 970 Mb, with an N50 of 691 Kb and 2549 contigs. These last two statistics indicated better contiguity compared to the other assemblers tested. The associated haplotype contigs rose from 109 Mb after assembly, to 320 Mb after unzipping, and dropped to 264 Mb after polishing. Removal of under-collapsed haplotypes reduced the genome size to 668 Mb across 1210 contigs with an N50 of 951 Kb. BUSCO (embryophyte) reported 94.8% complete, but with a somewhat high percentage of duplication (11.7%).

142

143 The FALCON primary assembly for *A. negundo* was also consistent in size across pipeline
144 stages, with a total length matching estimates at 481 Mb across 1481 contigs with an N50 of 625
145 Kb. Removal of haplotype duplication from the primary assembly decreased the overall length to
146 442 Mb, number of contigs to 1063, and increased the N50 to 700Kb. The BUSCO score was
147 94.1% with only 5.8% duplication.

148

149 ***Hi-C scaffolding***

150 The FALCON assembly was selected over Flye due to the substantially more contiguous
151 assembly it produced for *A. saccharum*, though it should be noted that the statistics for both
152 assemblers were comparable for *A. negundo*. Hi-C reads provided 65x coverage of the *A.*
153 *saccharum* genome. The final assembly was 626.33 Mb in 388 scaffolds with an N50 of 45.72
154 Mb and GC% of 35.7%. The 13 pseudo-chromosomes represented 97% of the genome length,
155 and BUSCO (embryophyte) scores were 97.7% complete with 3.0% duplicate, 0.7% fragmented,
156 and 1.6% missing.

157

158 *A. negundo* Hi-C reads provided 100x coverage and the FALCON assembly was used for
159 scaffolding due to potential mis-assembly in the Flye version ([Figure S2](#)). The final assembly
160 was 442.39 Mb in 108 scaffolds with an N50 of 32.30 Mb and GC% of 34.1%. The thirteen
161 pseudo-chromosomes represented 99.74% of the total length. BUSCO embryophyta scores were
162 97.4% complete with 5.6% duplicate, 0.9% fragmented, and 1.7% missing. ([File S1](#)).

Figure 2. Results of assembly testing with *A. saccharum*, comparing fragmentation in terms of total contigs versus assembly length. The dashed line represents the estimated genome size. Gray dots are short-read assemblers, shown as highly fragmented. Blue dots are long-read tests of assembly workflows. Canu refers to the use of reads error-corrected by the Canu pipeline. The red dot is the selected draft assembly, and the green dot shows scaffolding results following Hi-C. Detailed assembly statistics are available in [File S1](#).

Genome annotation

Annotations for *A. saccharum* resulted in 40,074 gene models of which 8,765 were monoexonics verified by the presence of a protein domain and start and stop codons. Transcriptome comparison based on 15,234 transcript loci supported 13,997 gene models. Functional annotation was applied via similarity search or gene family assignment for 35,304 models. *A. negundo* had 30,491 genes, 5,558 of which were monoexonic, and 16,168 transcript loci supported 14,682 of

177 the *de novo* models. Functional annotations were determined for 27,077 of the models. ([File S2](#)).
178 *A. saccharum* repeat content was at 64.4% while *A. negundo* was 58.6%.

179

180 Whole genome duplication and Acer synteny

181 Categorization of putative paralogs revealed a higher percentage of each type in *A. saccharum*
182 relative to *A. negundo*. Plots of Ks distribution for WGD genes in syntenic regions show a single
183 clear peak at a Ks range consistent with the core eudicot WGT reported in other species using the
184 same pipeline ([Figure 3a](#)). *A. yangbiense* does not have a recent WGD, and when compared to *A.*
185 *saccharum* which had an additional small recent peak, further investigation identified small
186 blocks of collinearity, a minimum of five genes in palindromic or tandem arrangements. These
187 blocks are predominantly located on a few scaffolds and are not reflective of the general
188 distribution typical of WGD ([File S3](#)). Macrosynteny analysis found that *A. negundo* and *A.*
189 *yangbiense* are syntenic, while comparisons between each of these and *A. saccharum* revealed a
190 large-scale translocation where two chromosomes from *A. negundo*, including the largest, are
191 split with sections exchanged to form two different chromosomes in *A. saccharum* ([Figure 3b](#),
192 [Figure S4](#)).

193

Figure 3. Ks distribution for WGD synteny blocks with a summary of duplication types in (a) *A. negundo* and (b) *A. saccharum*. Abbreviations for categories of duplication: WGD, whole genome duplication; TD, tandem duplication; PD, proximal duplication; TRD, transposed duplication; DSD, dispersed duplication. (c) Circos plot of the thirteen chromosomes ordered largest to smallest for *A. negundo* (orange bars) and *A. saccharum* (blue bars) with distributions of gene density (green) and transposable element frequency (purple). Syntenic regions are linked in gray with darker shades to visually highlight larger recombinations.

Expression analysis of *A. saccharum* aluminum and calcium treatments

The final annotated genome for *A. saccharum* served as a reference for the expression study. In total, there were 245 unique differentially expressed genes with 181 informatively described by sequence similarity descriptors. Of those with no similarity match, four were completely novel with no identifiable protein domain. Initial analysis produced six up and nine downregulated

207 genes comparing the aluminum to calcium treatments, and the other pairwise comparisons had
 208 similarly small totals. Clustering of the expression results showed season had a strong effect
 209 ([Figure S3](#)), so the analysis was repeated for each season individually to remove this variable
 210 from treatment comparisons. For brevity, abbreviations are used according to the following
 211 definitions: All, across seasons; Fa, fall; Sp, spring; Al, aluminium; Ca, calcium; and Un,
 212 unamended. FaAl to FaCa had 26 upregulated and 17 down, FaAl to FaUn had 7 up and 12
 213 down, and FaUn to FaCa had 28 up and 27 down. SpAl to SpCa had 39 up and 19 down, SpAl to
 214 SpUn had the greatest number with 33 up and 41 down, and SpUn to SpCa had 28 up and 11
 215 down. ([Figure 4](#), [File S4](#))

Figure 4. a) Differential expression study design showing number of samples collected in fall and spring from treatment plots at the Hubbard Brook Experimental Forest, Nutrient Perturbation

219 study. b) Differentially expressed genes (up and downregulated) for each treatment and season
220 comparison. Charts display both significance and relative expression denoted as log-fold change.
221 Dotted lines indicate thresholds of significance (0.1 p-adjusted, 1.5 log₂ fold change).

222

223 There were only two instances where the same gene was found in both separate seasonal
224 analyses. Transcription factor-like E2FE was upregulated in both unamended and aluminum
225 treatments in fall, spring, and all. It had the most isoforms expressed, and was also the most
226 highly upregulated for fall (8-fold). E2FE represses endoreduplication, reducing this type of
227 growth in response to stress (Hendrix et al., 2018). The second instance was disease resistance
228 protein At4g27190-like, expressed primarily in SpAl, but also FaUn and FaCa. Another
229 At4g27190 isoform was upregulated in SpCa and SpAl, but downregulated in SpUn.

230

231 GO enrichment results for fall showed sugar and carbohydrate transmembrane transporter
232 activity upregulated in the FaUn compared to FaCa ([Table S3](#)). Several sugar transporters,
233 SWEETs and ERD6-like, were seen in both seasons, though more often in fall. There were two
234 SWEET15 (tandem duplicates), one upregulated in FaUn to FaCa, and SWEET15-like
235 upregulated in SpUn relative to both SpCa and SpAl (7.6-fold). SWEET2a was upregulated in
236 FaAl to FaCa. Three different ERD6 were upregulated in FaUn with a fourth in SpAl.
237 Triterpenoid biosynthetic process was also enriched FaUn to FaCa, supported by two
238 downregulated beta-amyrin synthase genes, one of which was significantly downregulated even
239 further in FaAl to FaUn (5.4-fold). Other strongly differentiated genes included accelerated cell
240 death 6-like (ACD6) upregulated in FaCa (5-fold) relative to FaAl. It is associated with
241 flavin-containing monooxygenase 1 (FMO1), the most highly upregulated DEG in FaUn
242 (22-fold) and FaCa, both relative to FaAl.

243

244 In spring, DEGs upregulated in SpUn compared to SpAl were enriched in processes related to
245 biotic and abiotic stress, including gene ontology terms for defense response, and acid,
246 oxygen-containing, and antimicrobial response. There were six disease resistance genes, all
247 largely in SpUn and SpCa compared to two in fall. Also present in large numbers, were

248 serine/threonine kinases, including LRR receptor-like, with seven out of twelve of these in the
249 FaUn. Heat shock proteins were also common, though more equally split between the two
250 seasons and shifted toward unamended or aluminum. There were two copies of ITN1, involved
251 in salicylic acid signaling, one of which was the most upregulated DEG in SpCa to SpAl
252 (23.6-fold). In addition to direct stress response, there was an interesting increase of expression
253 in three Holliday junction resolvases, and a lamin-like gene, which was the second highest DEG
254 in these comparisons (19.7-fold). It can play a role in nuclear membrane integrity, chromatin
255 organization, and gene expression (Hu et al., 2019).

256

257 *Metal tolerance via ligation, sequestration, and transport*

258 In SpAl, upregulation of both metallothionein-like 3 (6.6-fold), and aluminum-activated malate
259 transporter (ALMT) 10 (4.5-fold) was observed. An EH domain-containing gene (4.6-fold) is
260 involved in endocytosis, vesicle transport, and signal transduction (Naslavsky & Caplan, 2005),
261 and several cytoskeletal related genes were seen such as kinesin in fall (6.8-fold), and myosin-6
262 in spring (8-fold).

263

264 In addition to the sugar transporters, ABC transporters were present as multiple isoforms in
265 FaUn, and one was the most upregulated DEG in FaUn compared to FaCa (13-fold). Two were
266 downregulated in FaAl compared to FaCa, but still elevated in FaUn, and were from family C
267 which contains pumps for glutathione S-conjugates, which have been shown to remove cadmium
268 in *Arabidopsis* (Tommasini et al., 1998). Additional transport included upregulation of ATPases
269 across seasons, cyclic and mechanosensitive ion channels in spring and fall, respectively, and a
270 K⁺/H antiporter upregulated in FaAl.

271

272 *Calcium dependent proteins*

273 Three calcium-transporting ATPases were upregulated in either FaCa or FaUn along with an
274 unspecified plasma membrane ATPase. Ca-based external signal relay mechanisms upregulated
275 in SpCa included a cyclic nucleotide-gated ion channel, G-type lectin S-receptor like
276 serine/threonine-protein kinases, and a glutamate receptor (5.5-fold) (Sun et al., 2013; Toyota et

277 al., 2018). A calcium-dependent protein kinase (4.5-fold) was upregulated in FaUn.
278 Calmodulin-binding proteins (60d at 5-fold) were up-regulated in both SpAl and SpCa, and are
279 associated with salicylic acid synthesis and immunity in *Arabidopsis* (Li et al., 2021).

280

281 *Reactive oxygen species response*

282 Antioxidant and redox genes included glutathione peroxidase, upregulated in FaUn, and
283 glutathione S-transferase, upregulated in both FaCa and SpAl. Two cytosolic sulfotransferases
284 and six thioredoxin genes were upregulated in SpAl. Other redox related genes include a highly
285 expressed epoxide hydrolase (9-fold) and a carotenoid cleavage dioxygenase (4.6-fold) in FaAl;
286 and peroxidase, aldehyde dehydrogenase (6.7-fold), and germin in SpAl. Ascorbate-dependant
287 oxidoreductase SRG1 was upregulated in SpUn, cytochrome P450 71D11 (a monooxygenase) in
288 SpAl, and zinc finger (C2H2 type) in SpUn.

289

290 *Cell wall and membrane integrity*

291 MDIS1-interacting receptor kinase, upregulated in FaUn relative to FaAl, and expansin, FaUn to
292 FaCa, are associated with the cell wall. Two ADP-ribosylation factor GTPase-activating, one of
293 which is the most highly upregulated gene in spring (19.9 fold-change SpAl to SpCa), assists
294 with cell signaling by recruiting cargo-sorting coat proteins at the membrane and regulating lipid
295 composition in support of development and defense (Donaldson & Jackson, 2011).

296

297 *Hormone crosstalk*

298 Auxin was indicated by three WAT genes (seen in each season, greatest at 7-fold in SpAl to
299 SpCa) and indole-3-acetic acid (highest of AllAl to AllUn at 9.7-fold). Two ethylene synthesis
300 genes were present: 1-aminocyclopropane-1-carboxylate oxidase, FaAl to FaCa, and
301 methylthioribose kinase, SpAl to SpCa. Ent-kaurenoic acid oxidase is related to brassinosteroid
302 homeostasis and gibberellin biosynthesis (Helliwell et al., 2001) and was present in SpAl to
303 SpUn (7-fold), and obtusifolios 14 α -demethylase, which mediates brassinosteroid synthesis, was
304 upregulated SpUn to SpCa (Xia et al., 2015). Jasmonic acid activity was upregulated in FaCa,
305 negative regulation of cytokinin was up in AllAl to AllUn, and ABA-induced HVA22 which

306 inhibits gibberellin and is possibly involved in vesicular traffic, was in SpAl to SpCa (8-fold) as
307 well as AllAl to AllCa.

308

309 ***Gene family evolution with expression study integration***

310 By leveraging 22 high-quality plant genomes, gene family dynamics between *A. negundo* and *A.*
311 *saccharum* revealed distinct characteristics. Comparisons among the plant proteomes resulted in
312 20,234 orthogroups with a mean size of 26.4 genes. Of these, 4,262 were shared by all species,
313 and 79 were single-copy. 88.5% of 603,640 genes were contained in orthogroups, and 0.7% were
314 species-specific. All species had at least 80% of their genes contained in orthogroups with the
315 exception of *Ginkgo biloba*, *Nymphaea colorata*, and *Oryza sativa* ([Table S4](#)).

316

317 All three *Acer* shared 11,156 orthogroups. *A. negundo* and *A. saccharum* had the largest overlap,
318 *A. saccharum* and *A. yangbiense* had the second largest overlap, and *A. saccharum* had the most
319 unshared groups. ([Figure 5b](#)). Comparing *Acer* against the other woody angiosperms (*B.*
320 *pendula*, *C. papaya*, *C. clementina*, *C. sinensis*, *E. grandis*, *J. hindsii*, *J. regia*, *P. vera*, *P.*
321 *trichocarpa*, *P. persica*, *Q. lobata*, *Q. robur*, *T. grandis*), 728 *Acer* orthogroups were expanded,
322 14 were contracted, 1992 were novel, and *Acer* was estimated to be missing from 202
323 orthogroups. To clarify, these may not be fully absent, but didn't have representation in
324 orthogroups including those that were more lineage specific. Comparing the Sapindales to the
325 other trees resulted in 340 expanded, 4 contracted, 2788 novel, and 160 missing. Dynamics in
326 common between the two *Acer* included 270 expanded groups, 1 contracted, 0 novel, and 247
327 missing ([File S5](#)).

328

329 *Acer* families expanded among the woody angiosperms were enriched for RNA modification,
330 DNA replication strand elongation, and processes of organic cyclic compound, cellular aromatic
331 compound, and heterocycle metabolisms. Novel genes were highly enriched for cell periphery
332 localization and marginally for sesquiterpenoid and triterpenoid biosynthesis ([Figure 5a](#), [Table](#)
333 [S5](#)). When focusing on absent gene families, none were found to be missing exclusively in
334 Sapindales, which includes the two *Citrus* species and *Pistacia vera*. A total of two families were

absent in all *Acer*, phosphatidylcholine transfer protein-like and cellulose synthase interactive 3, but were present in all other species.

337

Several interesting gene families more novel to *Acer* overlap with the HBEF DEGs. There are twenty orthogroups associated with disease resistance At4g27190, two seen as DEGs, and the specific families containing these DEGs are larger for *A. saccharum* with one novel to the *Acer*. Two additional non-DEG families are rapidly expanding in *A. saccharum*, with one of these also expanding in *A. negundo*, but both contracting in *A. yangbiense*. In fall, another more novel DEG ACD6-like belongs to a family with limited species membership (Sapindales and *V. vinifera*). Compared to *Arabidopsis* ACD6, which has two 3-repeating ankyrin domains, the *A. saccharum* ACD6-like had varying ankyrin positions, as do other members of this family. A third example, seen only in spring, acetyl-coenzyme A synthetase (ACS) is a member of a novel *Acer* family consisting of 1 *A. negundo*, 6 *A. saccharum*, and 18 *A. yangbiense*. Comparison with *A. negundo* and ACS isoforms reveals the *A. saccharum* gene contains a longer ACL domain, with only ~67% query coverage and ~43% percent identity to *A. negundo* which is much more similar to other ACS (87-90% length; ~88% identity).

351

Within the broader set of species, *A. saccharum* gene families were characterized by more expansion, with 1827 expanded, 18 contracted, 127 novel, and 511 absent, and more rapidly expanding, with 99 compared to 18 contracting. *A. negundo* had 1068 expanded, 23 contracted, 89 novel, and 558 absent orthogroups. Rapidly contracting families were greater in this species with 52 compared to 26 rapidly expanding ([Figure 5](#), [File S5](#), [File S6](#)).

357

A. saccharum gene families

Functional enrichment of *A. saccharum* in the full species comparison revealed that expansions were processes of ncRNA metabolism, RNA modification, organic cyclic compound metabolism, heterocycle metabolism, and intracellular membrane-bound organelle localization ([Table S5](#)). Almost half of the *A. saccharum* families had limited annotation information, due to either missing descriptors or uncharacterized protein matches. Relative to *Acer*, *A. saccharum*'s

364 expanded families are enriched in a larger list of stress response associated functions that are
 365 fairly specific, including water deprivation, hypoxia, salinity, heat, cold, xenobiotic, nematode,
 366 karrikin, acid chemical, and hormone ([File S13](#)). Other significant processes include regulation
 367 of indolebutyric acid stimulus (auxin family), RNA splicing, chloroplast RNA processing,
 368 phospholipid translocation, brassinosteroid homeostasis, lignin synthesis, microsporogenesis,
 369 phenylpropanoid biosynthesis, cadmium ion transmembrane transport, cyclin-dependent
 370 serine/threonine kinase, and calcium-transporting ATPase. Rapidly expanding families are
 371 associated with various biotic and abiotic responses, such as fungal, salt stress, and xenobiotic
 372 response ([File S6](#)). Interesting genes include patatin-like 2, involved in membrane repair via
 373 removal of lipids modified by oxidation (Yang et al., 2012) and ALP1 negative regulation of
 374 polychrome group chromatin silencing (Liang et al., 2015). Those that overlap with HBEF DEGs
 375 include disease resistance At4g27190 and DSC1, FMO1, rapidly expanding SRG1, and rapidly
 376 contracting disease resistance At1g50180.

377

378 Compared to other *Acer*, contracted families are enriched for pollen wall assembly, extracellular
 379 matrix assembly and organization, chlorophyll binding, NADH dehydrogenase (ubiquinone)
 380 activity, DNA-directed 5'-3' RNA polymerase activity, programmed cell death, and myb-like
 381 transcription factors ([File S11](#), [File S12](#)). Genes absent in *A. saccharum* but present in all other
 382 species total 28 ([File S15](#)), including red chlorophyll catabolite reductase (ACD2) and
 383 S-adenosyl-L-homocysteine hydrolase (HOG1), which is necessary to hydrolyze the by-product
 384 of the activity of S-adenosyl-L-methionine-dependent methyltransferase, and one of these was
 385 also absent. The apparent absence of HOG1 requires further investigation as mutants display a
 386 number of problematic phenotypes and variants display association with fiber length in *P.*
 387 *tomentosa* (Du et al., 2014).

388

389 *A. negundo* gene families

390 In the broad comparison of species, *A. negundo* expanded families were enriched in RNA
 391 modification, microgametogenesis, and metabolic processes of nucleobase-containing
 392 compound, organic cyclic compound, heterocycle, and cellular aromatic compound ([Table S5](#)).

393 The small number of rapidly expanding families were by far mostly uncharacterized proteins or
394 missing sequence similarity descriptions with only four out of 26 genes having a description,
395 including glutathione-S-transferase, two disease-resistance proteins, At4g27190-like and
396 At5g66900, a receptor-like 12, and additional functional descriptors such as E3 ubiquitin-protein
397 ligase, LRR receptor-like ser/thr kinase, and more ([File S6](#)). Relative to other *Acer*, *A. negundo*'s
398 expanded families are enriched in a short list of specific stress response including UV, UV-B,
399 radiation, bacterium, cadmium, metal ion, drug, chemical, and osmotic stress ([File S9](#), [File S10](#)).
400 Other processes include proanthocyanidin biosynthesis, lignin synthesis via cinnamyl-alcohol
401 and sinapyl-alcohol dehydrogenase, starch metabolism and glucan catabolism, error-prone
402 translesion synthesis, and other DNA damage response and repair. Processes related to
403 reproduction were present, especially pollen development. For example, decreased size exclusion
404 limit 1 (DSE1, aka aluminum tolerant 2 (ALT2)) is a transcription factor that regulates the size of
405 molecules that can travel through plasmodesmata, as channel aperture is not static, changing in
406 response to stress and decreasing during embryo development (Xu et al., 2012). DSE1 is single
407 copy in all species with expansions only in *A. negundo*, *Q. rober*, and *T. grandis*.
408
409 Contractions relative to other *Acer* include transcription by RNA polymerase III, chloroplast
410 RNA processing, and lignin biosynthetic processes ([File S7](#), [File S8](#)). Rapidly contracted
411 families were enriched in quercetin 3-O- and 7-O-glucosyltransferase activity ([Table S6](#)).
412 Examples of contracted families include 7-ethoxycoumarin O-deethylase, which metabolizes a
413 wide range of xenobiotics (Robineau et al., 1998), and disease resistance-like protein DSC1, both
414 rapidly expanding families in *A. saccharum* ([File S6](#)). There are 23 genes absent in *A. negundo*
415 that are present in all other species ([File S14](#)). Several of these are curious, as they appear to be
416 required components of important processes, such as AUGMIN subunits 2 and 7 that help form a
417 complex that plays a role in spindle microtubule generation (Tian & Kong, 2019), and cell
418 division cycle 45-like, which is required for meiosis in *Arabidopsis* (Stevens et al., 2004). The
419 absence of formamidopyrimidine-DNA glycosylase is also interesting as it is involved in base
420 excision repair of DNA damage, a notable area of specific enrichments described below.

Figure 5. a) Gene ontology enrichments for *Acer* (all three species combined), *A. negundo*, and *A. saccharum*. Abbreviations for gene family dynamics: E, expanded; N, novel; RC, rapidly contracting. b) Total gene families, shared and unique, among the *Acer*. c) Reconstructed gene tree showing contracted gene families in red and expanded in green.

Discussion

Completion of the first chromosome-scale genomes for two North American maples brings Sapindales to a total of 15 available reference genomes. Sequenced members in this group contain citrus, mangos, pistachio, and poison ivy. Sapindaceae is morphologically diverse and is known for having opposite leaves, colorful fall foliage, and samaras. The maple genus *Acer* represents over 150 species, most native to Eastern Asia and a small number in Eastern North America (10)

433 and Europe (22) (J. Li et al., 2019). Today, one out of five species are endangered in their native
434 range (Crowley et al., 2020) and will continue to face challenges from both abiotic and biotic
435 threats resulting from a rapidly changing climate.

436

437 At the trait level, both *A. saccharum* and *A. negundo* differ in distribution and tolerance to
438 abiotic stressors. *A. negundo* grows quickly, is able to reproduce after only five years, and has a
439 shorter lifespan of 60 years. It is moderately tolerant of a range of conditions and is widespread
440 throughout North America. In contrast, *A. saccharum* has slow growth until release of canopy
441 coverage, doesn't achieve reproductive maturity until 40 years, and is able to live 300 to 400
442 years. It requires high nutrient soils, prefers mesic environments, and is tolerant of cold, but not
443 salinity. Its range crosses a more narrow latitudinal, but stronger elevational, gradient, whereas *A.*
444 *negundo* tends to be more limited by elevation. *A. negundo* is considered an aggressive invasive
445 in Europe, South Africa, and parts of Asia and North America, and rapidly colonizes and
446 dominates disturbed habitats leading to loss of native species (CABI, 2021; Lamarque et al.,
447 2015). The sequenced individual in this study is from the native range where *A. negundo* is
448 highly plastic in growth, leaf unfurling, leaf mass area, maximum assimilation rate, as well as
449 nitrogen content and photosynthetic efficiency (Lamarque et al., 2015). *A. negundo* also exhibits
450 sexual dimorphism in photosynthetic rates, leaf size and allocation, and growth form, where
451 males are more successful in dry environments due to enhanced stomatal sensitivity and females
452 are found in more mesic environments (Dawson & Ehleringer, 1993). Invasive populations
453 maximize growth in high light and nutrients, with reduced performance in deficient conditions.
454 Even in optimal light, photosynthetic capacity and leaf nitrogen content remain low in *A.*
455 *negundo*. Such disadvantageous traits were surprising, and studies attributed its plasticity in
456 growth to morphology as increased leaf area allocation is a minimal investment allowing for
457 adjustment to changing conditions (Lamarque et al., 2013, 2015; Porté et al., 2011). A reciprocal
458 common garden study, examining invasive and native populations, found that in addition to
459 plasticity, post-invasion genetic differentiation was a factor in later stages of invasion success
460 (Lamarque et al., 2015). At least six varieties of *A. negundo* exist across the native range as well,
461 based on morphological characteristics (Rosario, 1988).

462

463 The maple genomes reveal support for their contrasting life histories. While both are small in size
 464 and diploid, *A. saccharum* is 42% larger, containing 38% more gene duplications, many very
 465 recent, and twice as many transposable elements. Gene families tend to be larger, more diverged,
 466 and undergoing rapid expansion in *A. saccharum* compared to *A. yangbiense* or *A. negundo*,
 467 which is characterized by contracting families, particularly among those rapidly evolving.
 468 The *A. negundo* reference genome is a small diploid with high heterozygosity and lower repeat
 469 (LTR) content. Synteny with *A. yangbiense* indicates there isn't much large-scale structural
 470 variation and supports its reduced character ([Figure S4](#)). Invasive plant species are often
 471 associated with smaller genomes. Traits such as fast growth rate, germination time, stomatal
 472 responsiveness, and dispersal ability are cell size or division rate dependent (Pyšek et al., 2018).
 473 The greater surface area to volume ratio of small cells, derived from small genomes, reduces the
 474 metabolic and signaling requirements, but does not preclude additional growth or activation, thus
 475 extending the range of capacity, or plasticity, for a wider set of traits (Roddy et al., 2019; Suda et
 476 al., 2015). The adaptive potential conferred by polyploidism can also be leveraged for invasion,
 477 and while polyploidism has not been documented in the native species, we cannot rule this out as
 478 a factor in *A. negundo*'s invasion success. The *A. negundo* genome also has a lower GC%
 479 relative to *A. saccharum*, and within phylogenetically close relatives, lower GC% is typically
 480 associated with smaller genomes. GC content indicates DNA base composition in terms of
 481 guanine and cytosine, which have different biochemical properties in base pair and higher order
 482 structure, nucleotide synthesis requirements, and methylation and mutation rates (Šmarda et al.,
 483 2014). Earlier work based on low coverage sequencing postulated they were at higher
 484 percentages, *A. negundo* in particular (Contreras & Shearer, 2018; Staton et al., 2015), but whole
 485 genome sequencing supports their place at the lower range among angiosperm plants (Trávníček
 486 et al., 2019). Higher relative GC% is associated with increased size, often as a result of increased
 487 LTR content and adaptation to colder climates or greater annual temperature fluctuations (Veleba
 488 et al., 2017). Extreme cold and/or desert environments are also conditions where lower
 489 metabolic rates might be selected for, accompanied by a larger genome (Roddy et al., 2019).
 490 While *A. saccharum* does not occupy extreme regions, it is found at higher elevations, and the

491 genome is enriched in cold and water-related response relative to *A. negundo*. In spite of *A.*
 492 *saccharum*'s larger size, GC%, and functional enrichments, it remains challenged by abiotic
 493 stress in its current range. It is similar in size and GC% to *A. yangbiense*, an endangered species
 494 found at high elevations in a very limited region of Yunnan Province (J. Yang et al., 2019). If
 495 larger genome size presupposes increased metabolic, transport, and nutrient demands (Pellicer et
 496 al., 2018) it is possible *A. saccharum*'s susceptibility to nutritional deficiencies, and calcium in
 497 particular, may be due to the extra burden of a larger genome and the various mechanisms
 498 within. The decrease in soil nutrient availability in its native range over the past decades is at
 499 odds with resources necessary to tolerate stressors brought on by a changing environment. From
 500 the foundational differences between *A. negundo* and *A. saccharum* in morphological,
 501 physiological, and genomic characteristics, we extend to the integration of gene family dynamics
 502 and expression data to further illuminate the contrasting strategies of competition versus
 503 resistance seen in these species.

504

505 *Herbivory, Reproduction, Light, DNA Damage*

506 Compared to the other *Acer*, *A. negundo*'s smaller set of response related functional enrichment
 507 is mainly limited to UV, bacteria, metal, cadmium, chemical, and osmotic response rather than
 508 the more extensive set seen in *A. saccharum*. Expression of these more specific response-related
 509 gene families could protect *A. negundo* from pests and pollutants, providing benefits conducive
 510 to life in an urban environment. A high portion of proanthocyanidin synthesis related genes were
 511 observed, which are precursors to condensed tannins that protect against herbivory, bacteria and
 512 fungal pathogens, and encroaching of neighboring plants (He et al., 2008). All four families were
 513 relatively novel with absence in all but one or two other species. Proanthocyanidins also have
 514 antioxidant and radical scavenging functions, so it would be interesting to see if these were
 515 differentially expressed in abiotic stress conditions similar to other flavonoids in *A. saccharum*.
 516 They have been compared to lignins in terms of pathogen defense mechanisms (Stafford, 1988),
 517 and some enrichment of expanded lignin precursor monolignol genes does exist, also assigned to
 518 relatively novel gene families.

519

520 Successful reproductive strategies are characteristic of invasive plants. Of the 62 reproduction
 521 related gene families expanded within *Acer*, over a third were also significantly expanded across
 522 the full set of species, with one third as pollen development, two thirds as fruit development, and
 523 a few related to embryonic development and seed germination. Pollen development related gene
 524 families included an ortholog of transcription factor DUO1, a key step in male germline
 525 specification that has been conserved yet heavily diverged in the evolution of male gametes
 526 (Higo et al., 2018). This expanded family is novel to the three *Acer* and contains multiple copies
 527 for the two dioecious species, with only one for *A. saccharum*. Within fruit development,
 528 LEUNIG, APETALA2-like (AP2), and another AP2 domain-containing family were
 529 significantly expanded, with yet a third AP2 domain-containing expanded within *Acer* for *A.*
 530 *negundo* but missing in *A. saccharum*. AP2 and LEUNIG are corepressors of homeobox gene
 531 Agamous, and in the absence of this repression, sepals and petals become stamens and carpels,
 532 as studied in *Arabidopsis* (Conner & Liu, 2000). The AP2 family is large, containing some
 533 members associated with germination, growth, and stress response (Krizek, 2015; Shu et al.,
 534 2018).

535
 536 Both species are enriched in gene families with DNA damage recognition and repair
 537 functionality, but *A. negundo* has a greater number, largely categorized as either error-prone
 538 translesion repair or meiosis-related. Within these are additional enrichments in regulation of
 539 leaf, seed, flower development, and post-embryonic structures, response to abiotic and biotic
 540 stress, and growth such as cell division and endoreduplication ([Figure 6](#)), all of which can be
 541 altered in response to DNA damage that can be UV, genotoxic, or oxidative in nature. UV
 542 damage can activate translesion synthesis, an error-prone repair mechanism designed to quickly
 543 eliminate lesions that might otherwise stall replication and lead to double-stranded breaks
 544 (Sakamoto, 2019). The mutation rate from this type of repair is much higher than others (Kunkel,
 545 2000) and depends on additional repair for correction. *A. negundo* has three families in this
 546 category expanded relative to *Acer*, two of which are significantly expanded among the full
 547 comparison of species. Metal toxicity, including Al^{3+} , also causes double stranded breaks
 548 resulting in inhibition of cell cycle progression and cessation of growth, notably in the root

(Zhang et al., 2018). The ATR gene family partially responsible for that type of cell cycle inhibition is actually larger in *A. saccharum*. This could contribute to decreased aluminum tolerance if expression is likewise increased. Too much DNA damage can initiate either programmed cell death or continuing growth without replication via endoreduplication, which is a way for plants to enlarge size via increased nuclear content without the usual cell division step, thus preventing the spread of heavily damaged DNA to new cells (Nisa et al., 2019).

Figure 6. *A. negundo* gene families with ontology related to DNA damage and repair, and secondary enrichments categorized by color. Circles with multiple colors indicate multiple ontology assignments. Lines indicated known or predicted interactions, or other association via text-mining, co-expression, or protein homology.

In addition to UV-related DNA repair, there is also potential adaptation for photooxidative stress. The early light induced protein 1 (ELIP) gene family stood out as significantly expanded in *A. negundo* relative to all other species, with twice as many genes compared to other *Acer*. Research has focused on ELIP's protection against high light (Huang et al., 2019), but it also

regulates seed germination in response to environmental factors (Rizza et al., 2011). ELIPs are expressed in roots, and in response to some abiotic stressors including nitrogen in *Populus* (Luo et al., 2015), indicating additional protective functions. Capacity for regulation of photosynthesis and DNA repair mechanisms, within a largely streamlined genome, could represent investments with large-scale effects, altering growth according to resources or stress as described for *A. negundo* in various nutrient and light conditions.

571

572 *Integration of A. saccharum expression studies*

In contrast to *A. negundo*'s plasticity and invasion success, many *A. saccharum* populations are facing maple decline due to limited tolerance of abiotic stress. Integration of gene expression sampling with gene family dynamics highlighted the genetic factors that may influence maple decline and adaptation. HBEF NuPert saplings grown in native ecological and environmental conditions within replicated long-term soil treatments provided an opportunity to examine differences in gene expression related to soil acidity, calcium deficiency, and increased aluminum availability. Previous experiments involving aluminum are typically short-term time series measurements of more immediate responses in very young seedlings maintained in controlled environments (Cardoso et al., 2019; Liu et al., 2009). Examination of these trees on the landscape allowed us to study the effects of long-term nutrient stress in conjunction with other life-history processes across seasons.

584

585 *Calcium is key for signaling*

A. saccharum requires nutrient-rich soils, and calcium is the common limiting element underlying maple decline. Increased acid deposition in regions of low base cation concentration limits calcium availability (Long et al., 2019; Schaberg et al., 2001, 2006; Sullivan et al., 2013), but trees improve long-term with the addition of calcium (Moore & Ouimet, 2021). Additional nutrient imbalances, such as magnesium, phosphorus, and potassium and nitrogen deficiency, as well as high aluminum and manganese concentrations, climatic effects, and biotic stressors such as defoliation, are compounding factors, culminating in widespread decline that has been studied throughout most of *A. saccharum*'s native range (Bal et al., 2015). The effects of calcium can be

594 studied using the HBEF NuPert plots, where calcium amendment was designed to recreate
 595 previous levels of soil availability. Annual amendments of CaCl_2 from 1995-1997 followed by
 596 applications of wollastonite in 1999 and 2015 ([Table S7](#)) resulted in a 50% increase of calcium in
 597 foliar tissues of maples, while also decreasing aluminum concentrations non-significantly. Trees
 598 in calcium plots devoted more carbon to growth than storage, were better able to flush after a late
 599 spring frost, produced more flowers, and increased seed germination (Halman et al., 2013).
 600 Calcium-dependent signaling mechanisms and abiotic stress are well studied in model species
 601 with recent work on homologous pathways in poplar and a wide variety of gymnosperm and
 602 angiosperm trees (Estravis-Barcala et al., 2020). Calcium transport and binding can activate and
 603 regulate primary components of stress response systems, including internalization of external
 604 signals, modulation of cytosolic levels for transient signaling, and lead to initiation and
 605 perpetuation of ROS responses, enzyme activation, and pathways of hormone modulation and
 606 secondary metabolism. Calcium and salicylic acid in combination can improve aluminum
 607 tolerance by increasing exudates, preventing root accumulation, decreasing root growth
 608 inhibition, and stimulating antioxidants as seen in soybean (Lan et al., 2016). The wide variety of
 609 processes, including genes such as Ca-transporting ATPases, calcium-dependent protein kinases,
 610 and calmodulin-binding proteins, were all important in the differential expression response and
 611 also observed in the gene family dynamics.

612

613 *Calcium is necessary for primary and secondary metabolic processes*

614 Seasonal differences between the differentially expressed genes were striking, revealing the
 615 effects of calcium treatments on the larger activities at work during the time of sampling.
 616 Additional calcium facilitates natural life cycle processes, while unamended treatments show an
 617 increase in stress response perhaps reflective of the additional burden of calcium deficiency
 618 combined with external stressors. In fall calcium treatments, genes involved in leaf senescence
 619 and remobilization of nutrients were highly expressed. The lack of other differential expression
 620 in this season tied to clear processes made it all the more interesting. ACD6 is active in both
 621 natural and stress response senescence and is calcium signaling dependent (Jasinski et al., 2021;
 622 Zhu et al., 2021). Given the context of where these genes were expressed, considering treatments

623 and other neighboring DEGs such as calcium-dependent protein kinase, the likelihood seems to
624 be the occurrence of natural leaf senescence in the presence of adequate calcium. The ACD6
625 isoform expressed here is quite varied from the *Arabidopsis* form, and its gene family
626 membership is limited to Sapindales. The FMO1 highly expressed in the same comparative
627 context is associated with ACD6 and senescence in other studies, and is also a new variant very
628 expanded in *A. saccharum*. FMO1 is a flavonoid antioxidant that appears in other aluminum
629 tolerance studies and is associated with auxin regulation in addition to cell death, reflecting the
630 multipurpose nature of these types of redox genes (Schlaich, 2007). Another senescence gene is
631 absent in *A. saccharum*, red chlorophyll catabolite reductase (RCCR aka ACD2), a non-green
632 chlorophyll degradation gene (Chen et al., 2019). The exact role of RCCR is unclear (Jockusch
633 & Kräutler, 2020), but it appears possible that it provides additional fitness advantages beyond
634 chlorophyll degradation, such as increased tolerance to infection (Mach et al., 2001). This,
635 combined with the ACD6 susceptibility to calcium deficiency, potentially creates complications
636 for nutrient reabsorption or other senescence related activities in fall that could affect spring
637 growth, development, and ability to respond to stress.

638

639 *Characterization of aluminum response in A. saccharum stem tissue*

640 Aluminum amendment in NuPert was designed to create the effect of future acidification. Al and
641 Ca compete for uptake by the root, so Al amendment and low Ca/Al molar ratios essentially
642 increase Ca deficient conditions. This is exacerbated by acid deposition which contributes to
643 leaching of base cations and increases the availability of more toxic forms of Al. The focus of
644 current literature on *A. saccharum* mortality, health, and regeneration is calcium depletion, with
645 improvements seen upon calcium amendment (Cleavitt et al., 2017; Huggett et al., 2007).
646 Previous studies examining physiological effects of aluminum versus calcium with a multi-tissue
647 study on mature *A. saccharum* found foliar levels of Al did not vary much by treatment and all
648 were well below thresholds of toxicity. Within this tissue, concentrations of Al dropped slightly
649 in Ca treatments, while Al treatments caused Ca to drop more strongly in dominant than
650 non-dominant trees (Halman et al., 2015). The main effects noted in aluminum treated trees were
651 moderate root damage and foliar antioxidant activity (Halman et al., 2013). Some studies from

652 other locations have also linked foliar Al and Ca levels (Schaberg et al., 2006) and stem Al levels
653 to branch dieback as seen in maple decline (Mohamed et al., 1997). Within the differential
654 expression analysis we can detect levels of stress response between the unamended and
655 aluminum treatments, as well as signs related to improved functionality with calcium
656 amendment.

657

658 Aluminum targets the cell wall, plasma membranes, DNA, RNA, and proteins. Accumulation
659 occurs primarily in the root, binding to pectin in the cell wall, increasing its rigidity and
660 decreasing permeability. Downregulation of cellulose and upregulation of callose, oxidative
661 stress, hormones, and DNA damage signaling all result in root growth inhibition (Sade et al.,
662 2016). The plasma membrane is another frequent target, where Al disrupts membrane potential
663 and membrane-bound solute transporters, affecting symplastic and apoplastic concentrations
664 (Kar et al., 2021). Damage within proteins and DNA reduces enzymatic activity and can trigger
665 cell cycle checkpoints. Plants can be susceptible, resistant, or tolerant of aluminum, and have
666 various abilities in avoidance or accumulation related to the different targets.

667

668 Genes specifically associated with aluminum resistance or tolerance were not present in the stem
669 tissue as they are typically expressed in the root, but upregulated genes related to metal transport
670 and sequestration are likely involved in aluminum remediation. Metallothionein 3 was highly
671 expressed in AllAl, though unevenly distributed throughout the replicates. Similar to other
672 metallothioneins, it works via ROS scavenging and metal ion homeostasis via chelation, while
673 potential transport and vacuolization capabilities are unknown (Hasan et al., 2017). In
674 salinity-tolerant *Oryza sativa*, this gene responded to cadmium, salinity, and oxidative stress
675 (Mekawy et al., 2018). Expression of growth inhibitors was another interesting finding. ACC
676 oxidase and IAA-amido synthetase are known to be interacting root growth inhibitors, and if
677 expression of these is elevated in the root as well, this would result in decreased tolerance in *A.*
678 *saccharum* regardless of other response mechanisms. Genes functionally adjacent to aluminum
679 resistance were present, such as CAMTA4. Its role in regards to aluminum is unknown, though it
680 is responsive to cold and stress-related hormones (Kidokoro et al., 2017), and CAMTA2 is a

681 positive regulator of ALMT1 (Tokizawa et al., 2015). ALMTs are anion channels involved in a
 682 wide variety of processes, and although ALMT1 is known to be expressed in roots in response to
 683 aluminum (Hoekenga et al., 2006), most ALMT are not thought to be involved in aluminum
 684 tolerance (Liu & Zhou, 2018). Recent characterization of ALMT10, upregulated in SpAl in a
 685 pattern similar to metallothionein, proposes involvement in homeostasis of Cl^- efflux and NO_3^-
 686 assimilation, induced by water deficit (Racero & J, 2020). ALMT9 is significantly expanded in
 687 *A. saccharum*, and though not a stem tissue DEG, it is thought to be a vacuolar malate channel
 688 involved in guard cell regulation (De Angeli et al., 2013).

689

690 Most of *A. saccharum*'s aluminum resistance families are modestly sized, with the exception of
 691 PEP carboxylase and a large family of mixed MATEs, with members likely similar to homologs
 692 studied in *P. trichocarpa* and *Arabidopsis* (N. Li et al., 2017). Overexpression of either of these
 693 increases efflux of organic acids at the root (Begum et al., 2009). The metallothionein,
 694 CAMTA4, and growth inhibitors ACC oxidase and IAA-amido synthetase were also expanded.
 695 This is in line with broader gene family comparisons which revealed fewer expanded aluminum
 696 resistance and tolerance families in some, but not all, species reported as low-tolerance
 697 phenotypes, including *C. papaya*, *P. persica*, and *V. vinifera* (Figure 7, File S16; Jaillon et al.,
 698 2007; Ming et al., 2008; Verde et al., 2017). Aluminum accumulator *A. trichopoda* was also
 699 contracted in these families, but accumulators specialize in Al sequestering and it is likely that
 700 other genes are involved (Jansen et al., 2002). Expanded families were seen more frequently in
 701 high-tolerance species such as *P. vera*, *P. trichocarpa*, *T. grandis*, *Q. lobata*, and *E. grandis*,
 702 though the mix of families varies (Figure 7; Q. Li et al., 2015; Sork et al., 2016; Tuskan et al.,
 703 2006; Zeng et al., 2019; Zhao et al., 2019). *A. negundo* had expansions related to tolerance that
 704 likely contribute to its ability to manage multiple agents of toxicity. Gene family size is only a
 705 partial view, for example, modification to promoter or intronic regions of Al resistance genes
 706 have been shown to increase or decrease the expression of organic acid efflux in barley, wheat,
 707 rice, and sorghum (Pereira & Ryan, 2019). Antioxidants and redox genes are also important
 708 factors of tolerance due to the increase in oxidative stress caused by internalized aluminum. It is

709 interesting that many of these DEGs are also from expanded families, in particular the
710 significantly expanded thioredoxins H3 and YLS8 and SRG1.

Figure 7. Orthogroup sizes for aluminum tolerance gene families are presented by species. Families were selected for inclusion based on documented aluminum tolerance and/or presence in the HBEF RNA-Seq differential expression results. Color represents the proportion of gene membership per species, with darker purple equating to more contracted families relative to the median, and dark green indicating expansion. (H) Family contains HBEF differentially expressed gene; (E) Expanded in *A. saccharum*; (C) Contracting; (M) Missing; (N) Novel; (*) Rapidly expanding; Categorization of tolerance is according to literature describing aluminum stress phenotypes. The undetermined category contains species where tolerance to aluminum or acidic soils has not been reported. ¹*B. pendula* is undetermined due to high variability in tolerance by genotype.

Contribution of flavonoids and specialist metabolites as antioxidants

Aluminum derived oxidative stress is a factor in inhibition of cell growth, and an early signal of aluminum toxicity (Yamamoto et al., 2003). Reactive oxygen species (ROS) are generated as a result of normal cellular processes, and reactive oxygen can take different forms, with varying toxicities. ROS is reduced by a variety of potential “scavengers” to prevent accumulation of levels that are damaging to lipids, proteins, DNA, and RNA, leading to cell death. ROS production and redox activity vary according to the primary processes of each cellular compartment (i.e., metabolism in mitochondria), and also in response to different stressors or stress combinations (i.e., drought and heat), so it is speculated that cells develop complex ROS signatures or hotspots that influence signal transduction and metabolic regulation in nuanced ways (Castro et al., 2021; Choudhury et al., 2017).

Transcriptomic studies of cadmium accumulators *Salix integra* and *Populus x canadensis* ‘Neva’ both expressed superoxide dismutase (SOD), with glutathione pathway genes and peroxidases, respectively (X. Li et al., 2021; Shi et al., 2016). Nickel stress in resistant versus susceptible genotypes of *Betula papyrifera* found GST and TRX in resistant trees (Theriault et al., 2016), and *A. rubrum*, a nickel avoider, had root-level expression of SOD, but downregulated GST (Nkongolo et al., 2018). In comparisons of aluminum treated *Citrus* root expression, peroxidases

741 and germin-like proteins were upregulated. The HBEF NuPert trees in aluminum plots were
 742 under oxidative stress that was at relatively low levels in controls. Halman et al. (2013) found
 743 elevated glutathione reductase in aluminum and ascorbate peroxidase higher in control and
 744 significantly so in aluminum. Similar to the other species, this transcriptomic study found
 745 antioxidants (TRX, two peroxidases, and a germin-like) all upregulated in response to the
 746 aluminum treatment, implying significant stress response activation. Aluminum increases
 747 peroxidation of lipids in membranes and produces H₂O₂ which can participate in retrograde
 748 signalling, regulating expression of additional genes (Castro et al., 2021). The expression in stem
 749 tissue implies that aluminum has translocated from the root to other tissues where it is causing
 750 peroxidation of membranes. Glutathione system members, which can also act as chelators, are
 751 more broadly seen in both unamended and aluminum, with one actually downregulated in Al
 752 similar to *A. rubrum*.

753

754 In calcium treatments, there are alternative forms of oxidative reduction, perhaps associated with
 755 different processes when sufficient levels of calcium are maintained. NADPH-dependent
 756 2-alkenal reductase, one of two DEGs seen in *A. saccharum*'s oxidative stress gene family
 757 enrichment relative to *Acer*, is associated with mediating photooxidative injury and improved
 758 photosynthesis, nutrient use efficiency, and biomass (Mano et al., 2005; Wang et al., 2021). It
 759 was very highly upregulated in AlUn and AlCa. There is also a variant zinc finger
 760 cysteine-2/histidine-2-type transcription factor. These are general transcription factors, but in *P.*
 761 *euphratica* stem tissue, it promoted the expression of an ascorbate peroxidase to scavenge ROS,
 762 which resulted in greater freezing tolerance while maintaining growth (He et al., 2019). Here, it
 763 was expressed in SpUn and to a lesser degree in SpCa, but was very low in aluminum, and is
 764 perhaps another example of calcium dependency, in this instance correlated with ROS stress
 765 response.

766

767 There are many multifunctional specialist metabolites, such as flavonoids, that assist with
 768 general membrane stability through ROS homeostasis, hormone crosstalk, regulation of stress
 769 response transcription, and protein modification (Arora et al., 2000). The cumulative effect of

770 their oxidative capacity via direct and indirect methods likely has a significant effect (Bartwal et
771 al., 2013) in any acclimation these trees experience due to long-term treatments. More ROS
772 response is found in spring samples as fits with the trend of increased overall activity in spring.
773 The extent and complexity of ROS response can also be seen in the *A. saccharum* DEGs and in
774 the variety gene families expanded and contracted in both *A. saccharum* and *A. negundo* relative
775 to each other. Both *Acer* have expanded redox gene families, but *A. saccharum* has more, and
776 there is no close functional similarity in various types seen between the two species. There are a
777 few DEGs that are members of expanded families, but they are mostly just functionally similar,
778 rather than specific overlaps between these two datasets.

779

780 *Intersection of redox, hormones, and growth in response to aluminum*

781 Expanded genes families with members also highly expressed in AllAl include several that
782 involve hormones. Highly elevated in both seasons and expanded relative to other *Acer*,
783 ent-kaurenoic acid is part of gibberellin biosynthesis and also brassinosteroid synthesis
784 (Helliwell et al., 2001). It is possible there are hormone related growth reductions in the highest
785 levels of aluminum. 1-aminocyclopropane-1-carboxylate (ACC) oxidase is a precursor of
786 ethylene biosynthesis. Indole-3-acetic acid (IAA) -amido synthase controls auxin homeostasis by
787 creating IAA-amino acid conjugates. It suppresses expansin, a cell wall modification gene which
788 loosens cell walls in preparation for growth (Daspute et al., 2017; Ding et al., 2008; Z.-B. Yang
789 et al., 2014). ACC-oxidase and IAA-amido synthase, known participants in aluminum-based root
790 inhibition, are both upregulated in AllAl, and expansin is upregulated in FaUn along with other
791 cell wall and growth related DEGs, such as a variant MDIS1-interacting receptor that increases
792 cell wall integrity maintenance. In both seasons there was also a very strong upregulation of cell
793 cycle regulator E2FE, which prevents increases in growth via cell size by halting
794 endoreduplication. This could be initiated by hormones originating from oxidative stress, but it is
795 possible the underlying issue is DNA damage caused by aluminum toxicity. Endoreduplication is
796 one way plants maintain growth without replicating damaged DNA, and such damage is
797 indicated by several Holliday junction resolvases which repair double stranded breaks (Adachi et
798 al., 2011). Cadmium toxicity reduces endoreduplication, so if aluminum has the same effect,

perhaps E2FE is part of the suppression mechanism. It is interesting that all these genes, ACC oxidase, IAA-amido synthase, expansion, ATR, and even the endoreduplication response have all been studied in the root, and here, in stem cells, there is some evidence of implication in non-root reductions of growth reported in aluminum treated trees.

Aluminum treatments and the acetyl co A / aldehyde dehydrogenase pathway in spring

Acetyl-coenzyme A synthetase (ACS) activates acetate to acetyl-coenzyme A, a key component of fatty acid metabolism and a source of acetyl moieties for many post-translational modifications and signals, such as lysine acetylation of histones and many of the differentially expressed proteins seen (X. Wu et al., 2011). Acetyl-coenzyme A is the product of several alternate, compartmentalized pathways. Pyruvate dehydrogenase complex (PDC), contained in the peroxisome, is the primary pathway for fatty acid synthesis, while ACS functions in the chloroplast. ACS produces fatty acids and Leucine, but not organic acids citrate, malate, and fumarate and other amino acids that tend to result from PDC (Binder, 2010; Fu et al., 2020). As an alternative pathway, it is important for acetate homeostasis, necessary for proper growth and development. ACS is in many ways redundant to PDC, but it has a different source of acetate, derived from ethanol. Ethanol is converted by alcohol dehydrogenase to acetaldehyde, and aldehyde dehydrogenase (ALDH) converts this to acetate. ACS and ALDH have similar patterns of expression in spring aluminum samples. This particular ACS is a member of a gene family novel to the *Acer* species and is considerably varied from *A. negundo*. The variation and expansion of the gene family combined with expression indicates there is a possibility it is under positive selection.

HBEF expression data profiles trees existing in long-term, chronically stressful conditions. While these are not controlled greenhouse studies on aluminum response or calcium deficiency, and it is possible the trees are responding to more than one stress condition or other variables, replicated samples reveal complex expression indicating multiple forms of stress response in aluminum treatments and also somewhat in unamended plots. Differentially expressed genes, often related to signaling, transport, redox, hormones, and growth, are not seen as extensively in

calcium treatments, and furthermore, there is enhancement of other processes, such as senescence, and disease response in calcium. With this data, we provide molecular support to the many studies correlating maple decline with calcium-poor soils exacerbated by acidity, including a 30-year study showing extensive regenerative failure (Cleavitt et al., 2017). Given the lower level of foliar aluminum seen in dominant sugar maple at HBEF, and large amount of variability in aluminum response even between conspecific genotypes, further transcriptomic studies of root tissue would be beneficial for a better understanding of aluminum resistance mechanisms. A number of the differentially expressed genes also seem to be novel or highly diverged, and would benefit from further functional analysis in *Acer*. Higher elevations may become a climatic refugia, and identification of genotypes better adapted to base cation depleted soils may become increasingly important.

Conclusion

In this study we present new chromosomal-length *Acer* genomes for *A. saccharum* and *A. negundo*. We conducted an expression analysis of *A. saccharum* subjected to long-term aluminum and calcium treatments, and identified many genes related to the abiotic stress response and calcium deficiency. Differential expression results from stem tissue were complex, but larger trends were revealed. Aluminum and unamended treatments had upregulated stress response indicating potential damage caused by Al. The necessity of adequate Ca was reflected in calcium treatments by an absence of the abiotic stress response seen at unamended levels, and an upregulation of normal processes, such as seasonal senescence and disease response activity. Gene families related to aluminum stress tolerance were compared between the two species, and showed moderate expansion of families in *A. saccharum*. Broader gene family comparisons revealed expansion in traits associated with invasiveness in *A. negundo*. Release of these genomes and a complementary expression analysis of trees in the HBEF NuPert study shed further light on mechanisms of tolerance to acidic soil conditions and potential adaptations of increasing importance due to climate change.

Methods

857

858 **Sequencing**

859 Leaf material for *A. saccharum* was collected from the University of Maryland College Park
 860 Campus (GPS location 38°59'16.0"N 76°56'32.8"W). *A. negundo* was sourced from the
 861 ForestGEO Forest Dynamics plot (tag # 91603) located in Smithsonian Environmental Research
 862 Center, Edgewater, MD. Leaves were dark treated for at least one day prior to collection and
 863 shipped to Amplicon Express Inc. (Pullman, WA, USA) for DNA extraction. Both species were
 864 sequenced with Pacific BioSciences Sequel v2.1 using a total of fourteen SMRTcells, with the
 865 SMRTbell® Express Template Prep Kit v1.0, insert size 40 Kb, library size selection of 66 Kb
 866 for *A. saccharum* and 55 Kb for *A. negundo*. The resulting short read sequencing consisted of
 867 two lanes of Illumina HiSeq 2500 150PE with insert sizes ranging from 500 to 600 bp
 868 (Genomics Resource Center, University of Maryland School of Medicine Institute for Genome
 869 Sciences, Baltimore, MD, USA). For Hi-C, DNA leaf material was collected from the same
 870 individuals and extracted with the Phase Genomics Proximo Hi-C Plant kit (Phase Genomics,
 871 Seattle, WA, USA). Sequencing consisted of two lanes of mid output Illumina NextSeq 500
 872 75PE with an average of 150 M reads per species (Center for Genome Innovation (CGI) at the
 873 University of Connecticut, Storrs, CT, USA). For the RNA-Seq used in annotation, leaf samples
 874 were collected from one individual per species at two years of age grown at the Michigan State
 875 University greenhouse. Sequencing consisted of Illumina HiSeq at 100PE.

876

877 Stem tissue from *A. saccharum* was sampled from nine trees across two seasons from the
 878 Nutrient Perturbation study plots at HBEF (North Woodstock, NH, USA). A single sapling (dbh
 879 < 21 cm) was selected from three plots in each of the three treatments (calcium, control,
 880 aluminum). A total of 18 libraries (nine in Oct 2017 (Fall); nine in May 2018 (Spring)) were
 881 sampled ([Table S2](#)). Tissue was sampled from five years of growth measured by spring wood
 882 internodes. Sections were frozen in liquid nitrogen in the field and RNA were extracted after
 883 grinding tissues in liquid nitrogen. Extractions were run on Agilent Bioanalyzer Tapestation for
 884 quantification and RNA integrity. Two samples were unsuccessful (one aluminum and one

885 calcium) from the spring sampling. Libraries were prepared using Illumina TruSeq Stranded
886 mRNA and sequenced with Illumina NextSeq 500, 150PE (CGI).

887

888 ***Draft genome assembly***

889 In advance of assembly, genome size was estimated with Jellyfish v2.2.6 (21-mers) and
890 GenomeScope (Vurture et al., 2017) using the trimmed Illumina short-reads. The *A. saccharum*
891 reads were test assembled using Illumina short-read, raw and corrected long-reads, and a hybrid
892 of both. Draft assemblies were evaluated in relation to expected genome size, contiguity (N50
893 and number of contigs), conserved seed plant orthologs, and genomic/transcriptomic read
894 alignments.

895

896 The best draft assemblies leveraged the deep PacBio sequencing (*A. saccharum* 103x, *A.*
897 *negundo* 141x) and prioritized assembling repetitive regions of the genome and resolving the
898 heterozygosity, found in both species. During this phase of the process, two draft assemblies of
899 comparable results were used to investigate scaffolding potential. One was created with
900 FALCON (pb-assembly v0.0.6) a *de novo*, diploid-aware assembler for PacBio (Chin et al.,
901 2016), and the other was done using Canu (v1.6) error-corrected PacBio reads as input (Koren et
902 al., 2017) to Flye v2.3.7, an efficient haploid assembler that leverages repeat graphs with read
903 alignment techniques to resolve areas of repetition (Kolmogorov, 2016/2019).

904

905 Alternate heterozygous contigs (haplotigs) were separated from the primary assemblies using
906 Purge Haplotigs v1.0.4 (Roach et al., 2018). To determine coverage, PacBio reads were aligned
907 to the primary assemblies with Minimap2 v2.15-r911-dirty (H. Li, 2018). RepeatModeler v1.0.8
908 was used to create the repeat libraries for this analysis.

909

910 ***Hi-C scaffolding***

911 Long-range scaffolding of the FALCON/Purge Haplotigs assembly with Hi-C reads followed
912 processes recommended for the following suite of tools (*Genome Assembly Cookbook*, 2019).
913 HiC reads were aligned with BWA mem -5SP and PCR duplicates removed with samblaster

914 v0.1.24 (Faust & Hall, 2014). Scripts from the Juicer v1.5.6 (Durand, Shamim, et al., 2016)
 915 pipeline were modified to identify the Sau3AI restriction enzyme. The resulting Hi-C alignment
 916 file was provided to 3D-DNA v180419 (Dudchenko et al., 2017) for scaffolding, leveraging
 917 different parameters for each species according to the differing draft assembly characteristics. In
 918 particular, --diploid was added to *A. saccharum* to address remaining under-collapsed
 919 heterozygosity. JuiceBox was used to visualize Hi-C mapping against each scaffold created by
 920 the different parameter tests to visually detect which incorporated the majority of the contigs into
 921 the expected 13 pseudo-chromosomes (Dudchenko et al., 2018; Durand, Robinson, et al., 2016).
 922

923 ***Genome annotation***

924 RepeatModeler v2.01 (Flynn et al., 2020) and RepeatMasker v4.0.6 (Smit et al., 2013) were
 925 used to softmask the assembly. Trimmed RNA reads were aligned to the assembly with Hisat2
 926 v2.1.0 (Kim et al., 2015). GenomeThreader v1.7.1 (Gremme et al., 2005) was used to align
 927 protein sequences (derived from *de novo* transcriptome assembly; -gcmcoverage 80,
 928 -dpmindexonlen 20 -startcodon -finalstopcodon -introncutout). Structural gene prediction was
 929 executed with BRAKER2 v2.0.5 (Hoff et al., 2016). The process converted RNA-Seq alignments
 930 to exon support in GeneMark-ET v4.32 (Lomsadze et al., 2014), and combined this output with
 931 protein alignments for two rounds of training with AUGUSTUS v3.2.3 (Stanke et al., 2006,
 932 2008; Camacho et al., 2009) to predict coding regions in the genome. Extensive filtering was
 933 performed on the predicted gene space using gFACS v1.1 (Caballero & Wegrzyn, 2019).
 934 Evaluation of structural annotations were conducted with BUSCO and the PLAZA CoreGF
 935 rosids database v2.5 (Van Bel et al., 2019; Veeckman et al., 2016). Transcriptomic alignments
 936 were used to identify where they fully overlapped BRAKER-based models or provided
 937 additional support to those that did not pass previous filtering criteria. Transcriptome assemblies
 938 were conducted *de novo* with Trinity v2.20 (Grabherr et al., 2011). EnTAP v0.8.0 (Hart et al.,
 939 2018) was used to frame-select, functionally annotate, and identify potential contaminants for
 940 filtering, including bacteria, archaea, opisthokonta, rhizaria, and fungi. The resulting translated
 941 protein sequences were clustered with USEARCH v9.0.2132 (Edgar, 2010) at an alignment
 942 identity of 0.90. Transcriptomic alignments created with GMAP v2019-06-10 (T. D. Wu &

943 Watanabe, 2005) and gFACs were compared against the genome annotation using GffCompare
944 v0.11.5 (Pertea, 2018).

945

946 Functional annotation was performed using EnTAP v0.9.1 (Hart et al., 2020), a pipeline that
947 integrates both similarity search and other annotation sources including gene family (eggNOG),
948 protein domains (Pfam), gene ontology, and pathway assignment (KEGG). The following public
949 databases were included: NCBI RefSeq Complete, EMBL-EBI UniProt, and *Arabidopsis*
950 (TAIR11) .

951

952 ***Comparative genomics***

953 The translated gene space of 22 plant species were used for gene family analysis. *Acer* included
954 *A. saccharum*, *A. negundo*, and *A. yangbiense*. The remaining species were selected from high
955 quality public annotations. Fourteen broadleaf trees (*Betula pendula*, *Carica papaya*, *Citrus*
956 *clementina* and *sinensis*, *Eucalyptus grandis*, *Juglans hindsii* and *regia*, *Pistacia vera*, *Populus*
957 *trichocarpa*, *Prunus persica*, *Quercus lobata* and *robur*, and *Tectona grandis*), plus one woody
958 angiosperm, *Vitis vinifera*, were included, along with one gymnosperm, *Ginkgo biloba*. *Oryza*
959 *sativa* Kitaake was the representative monocot, along with *Amborella trichopoda* and *Nymphaea*
960 *colorata*, representing other more basal lineages, and *Arabidopsis thaliana*, being the primary
961 plant model system. OrthoFinder v2.3.7 (Emms & Kelly, 2015, 2018) was used to generate
962 orthogroups. Resulting gene counts per orthogroup for *A. negundo*, *A. saccharum*, and the mean
963 of the combined three *Acer* were each compared to the mean of other species to identify
964 potentially expanded, contracted, missing, and novel gene families. The initial delineation of
965 expansion and contraction was set at 2-fold above the standard deviation. Full absence was
966 verified with alignment of the *Arabidopsis* protein sequence against the assembly. CAFE v5
967 (Mendes et al., 2020) was used to identify rapidly evolving gene families. Values from the
968 newick species tree produced by OrthoFinder were multiplied by 100 to prevent issues with
969 rounding in CAFE, and the tree was made ultrametric using OrthoFinder. The poisson root
970 frequency distribution was run three times on gene families filtered by size to ensure
971 convergence of a lambda value representing birth and death rate. The selected lambda value was

972 then used to run the large family set. Functional enrichment of the resulting families was
973 obtained from gProfiler v:e99_eg46_p14_f929183 (Raudvere et al., 2019) using the annotation
974 of the representative (longest) gene when aligned to *Vitis vinifera*. This well annotated woody
975 angiosperm represents an ideal source having no recent WGD (Tang et al., 2008).

976

977 ***Whole genome duplication and syteny analysis***

978 Characterization of putative paralogs, including whole genome duplication was done as
979 previously described (Qiao et al., 2019) using DupGen_finder to separate whole genome,
980 tandem, proximal, transposed, and dispersed duplicates. Categorization can be helpful in
981 speculating on the origin of duplication such as transposable element activity, localized
982 replication error, larger segmental translocations, or ploidy events. The whole genome duplicate
983 frequency distribution was plotted by Ks value for analysis of peaks. Microsyteny of the small
984 peak of recent supposed whole genome duplication seen in *A. saccharum* was further analyzed
985 with MCScanX-based collinearity scripts (Nowell et al., 2018), as well as overall macrosyteny
986 between the three *Acer*.

987

988 ***Differential expression analysis***

989 HBEF NuPert plots were used as a basis for this study as they were designed to reflect acidity
990 and calcium levels over time as described by Berger et al. 2001. They consist of 12 *A. saccharum*
991 dominant plots near reference watershed 6, with four receiving annual AlCl_3 treatments 12 times
992 from 1995 to 2015, and CaCl_2 treatments were applied to 4 other plots for 4 years, followed by
993 applications of slow-release wollastonite in 1999 and 2015 ([Table S7](#)). Three samples were
994 collected from aluminum, calcium, and unamended control plots as described in the Sequencing
995 section. Trimmed reads for each of the sixteen successful HBEF-sourced libraries were aligned
996 to the *A. saccharum* reference genome with Hisat2 v2.1.0, and read counts were extracted with
997 htseq-count v0.11.2. The R Bioconductor package, DESeq2 v1.26.0 (Love et al., 2014), was used
998 for the expression analysis with the calcium as the control in pairwise comparisons of
999 unamended to calcium and aluminum to unamended, representing increasing levels of aluminum,
1000 and then aluminum to calcium, contrasting the extremes. P-adjusted values greater than 0.1 were

1001 filtered. Pairwise comparisons, specific to each season (fall and spring) as well as combined
1002 resulted in a total of nine sets. Gene Ontology (GO) enrichment was conducted with g:Profiler
1003 (database version e99_eg46_p14_f929183; Raudvere et al., 2019) using alignments of
1004 differentially expressed protein models to both *Vitis vinifera* (Phytozome v12.1) and *Arabidopsis*
1005 *thaliana* (TAIR11) as baseline annotations.

1006

1007 *A. thaliana* was used as a representative model for pathway analysis in the Genemania
1008 application for Cytoscape v3.8.0. and *V. vinifera* (NCBI taxon ID:29760) (Franceschini et al.
1009 2013) was used similarly with STRINGDB v.11 in Cytoscape v2.7.1. Differentially expressed
1010 proteins for each pairwise comparison were used to visualize the fold-change values in context
1011 of the supported pathways. Networks were constructed with a confidence score of 0.4 and 20
1012 maximal additional interactions (Shannon et al. 2003) and additional networks for protein
1013 models reported to be responsive to calcium deficiency and aluminum biotoxicity were imported
1014 and merged.

1015

1016 **Data Availability**

1017 Sequencing for *A. negundo*, along with the genome, annotations, and RNA-Seq are available in
1018 BioProject PRJNA750066. Corresponding data for *A. saccharum* is in BioProject
1019 PRJNA748028 with the exception of RNA-Seq used in annotation which is available in
1020 PRJNA413418. RNA-Seq used in the differential expression study are available in BioProject
1021 PRJNA751902. Full details on assembly, annotation, gene family analysis, and differential
1022 expression analysis can be found at <https://gitlab.com/PlantGenomicsLab/AcerGenomes>.

1023

1024 **Acknowledgements**

1025 NGS was funded by the National Science Foundation (NSF DEB-2029997; NSF EF-1638488).
1026 Hi-C library preparation and sequencing was funded by the Ronald Bamford Fund Endowment
1027 for Ecology and Evolutionary Biology to the Department of Ecology and Evolutionary Biology,
1028 University of Connecticut. Support for the HBEF RNA-Seq was provided by the University of
1029 Connecticut Center for Biological Risk.

1030

1031 The authors would like to thank the Institute for Systems Genomics (ISG) at UConn for
1032 sequencing support, the Microbial Analysis Resources and Services (MARS) within the Center
1033 for Open Research Resources & Equipment (COR²E) at UConn for RNA extraction support, the
1034 Computational Biology Core for HPC services, and the Hubbard Brook Experimental Forest for
1035 access to the NuPert plots. Thanks to Laura Figueroa Corona for assistance with the range map,
1036 and Sumaira Zaman, Alison Scott, and Nasim Rahmatpour for sharing computational approaches
1037 and scripts. Thank you to Dr. Yaowu Yuan for advice on both data analysis and the manuscript.
1038 The *A. negundo* individual sequenced is a part of the Smithsonian Environment Research Center
1039 Forest Dynamics Plot, which is part of the Smithsonian's ForestGEO network. We thank
1040 ForestGEO and Director Stuart Davies for encouraging tree genome research in the network and
1041 for facilitating and funding (NSF DEB-1046113; NSF DEB-1545761) as well as previous
1042 working group discussions on the topic.

1043

1044 **Author Contributions**

1045 SM, NS, JW, PS, and UU conceived and designed various aspects of the study; JW and NS
1046 coordinated and managed the study; SM, UU, NS, PS, AT performed the sampling and
1047 experiments; AT performed transcriptomic analysis; SM performed the assembly, annotation, and
1048 related comparative genomic and expression analysis under the advisement of JW; SM, JW
1049 wrote the manuscript; UU, NS, and AT contributed sections. All authors read and approved the
1050 final manuscript.

1051

1052

References

- Adachi, S., Minamisawa, K., Okushima, Y., Inagaki, S., Yoshiyama, K., Kondou, Y., Kaminuma, E., Kawashima, M., Toyoda, T., Matsui, M., Kurihara, D., Matsunaga, S., & Umeda, M. (2011). Programmed induction of endoreduplication by DNA double-strand breaks in *Arabidopsis*. *Proceedings of the National Academy of Sciences*, 108(24), 10004–10009. <https://doi.org/10.1073/pnas.1103584108>
- Arora, A., Byrem, T. M., Nair, M. G., & Strasburg, G. M. (2000). Modulation of Liposomal Membrane Fluidity by Flavonoids and Isoflavonoids. *Archives of Biochemistry and Biophysics*, 373(1), 102–109. <https://doi.org/10.1006/abbi.1999.1525>
- Bal, T. L., Storer, A. J., Jurgensen, M. F., Doskey, P. V., & Amacher, M. C. (2015). Nutrient stress predisposes and contributes to sugar maple dieback across its northern range: A review. *Forestry: An International Journal of Forest Research*, 88(1), 64–83. <https://doi.org/10.1093/forestry/cpu051>
- Bartwal, A., Mall, R., Lohani, P., Guru, S. K., & Arora, S. (2013). Role of Secondary Metabolites and Brassinosteroids in Plant Defense Against Environmental Stresses. *Journal of Plant Growth Regulation*, 32(1), 216–232. <https://doi.org/10.1007/s00344-012-9272-x>
- Begum, H. H., Osaki, M., Watanabe, T., & Shinano, T. (2009). Mechanisms of Aluminum Tolerance in Phosphoenolpyruvate Carboxylase Transgenic Rice. *Journal of Plant Nutrition*, 32(1), 84–96. <https://doi.org/10.1080/01904160802531035>
- Berger, T. W., Eagar, C., Likens, G. E., & Stinger, G. (2001). Effects of calcium and aluminum chloride additions on foliar and throughfall chemistry in sugar maples. *Forest Ecology*

- 1075 *and Management*, 149(1), 75–90. [https://doi.org/10.1016/S0378-1127\(00\)00546-6](https://doi.org/10.1016/S0378-1127(00)00546-6)
- 1076 Binder, S. (2010). Branched-Chain Amino Acid Metabolism in *Arabidopsis thaliana*. *The*
- 1077 *Arabidopsis Book / American Society of Plant Biologists*, 8.
- 1078 <https://doi.org/10.1199/tab.0137>
- 1079 Caballero, M., & Wegrzyn, J. (2019). gFACs: Gene Filtering, Analysis, and Conversion to Unify
- 1080 Genome Annotations Across Alignment and Gene Prediction Frameworks. *Genomics,*
- 1081 *Proteomics & Bioinformatics*, 17(3), 305–310. <https://doi.org/10.1016/j.gpb.2019.04.002>
- 1082 CABI. (2021). *Acer Negundo*. Invasive Species Compendium. <https://www.cabi.org/ISC>
- 1083 Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L.
- 1084 (2009). BLAST+: Architecture and applications. *BMC Bioinformatics*, 10(1), 421.
- 1085 <https://doi.org/10.1186/1471-2105-10-421>
- 1086 Cardoso, T. B., Pinto, R. T., & Paiva, L. V. (2019). Analysis of gene co-expression networks of
- 1087 phosphate starvation and aluminium toxicity responses in *Populus* spp. *PLOS ONE*,
- 1088 14(10), e0223217. <https://doi.org/10.1371/journal.pone.0223217>
- 1089 Castro, B., Citterico, M., Kimura, S., Stevens, D. M., Wrzaczek, M., & Coaker, G. (2021).
- 1090 Stress-induced reactive oxygen species compartmentalization, perception and signalling.
- 1091 *Nature Plants*, 7(4), 403–412. <https://doi.org/10.1038/s41477-021-00887-0>
- 1092 Chen, Z., Lu, X., Xuan, Y., Tang, F., Wang, J., Shi, D., Fu, S., & Ren, J. (2019). Transcriptome
- 1093 analysis based on a combination of sequencing platforms provides insights into leaf
- 1094 pigmentation in *Acer rubrum*. *BMC Plant Biology*, 19(1), 240.
- 1095 <https://doi.org/10.1186/s12870-019-1850-7>
- 1096 Chin, C.-S., Peluso, P., Sedlazeck, F. J., Nattestad, M., Concepcion, G. T., Clum, A., Dunn, C.,

1097 O'Malley, R., Figueroa-Balderas, R., Morales-Cruz, A., Cramer, G. R., Delledonne, M.,
1098 Luo, C., Ecker, J. R., Cantu, D., Rank, D. R., & Schatz, M. C. (2016). Phased diploid
1099 genome assembly with single-molecule real-time sequencing. *Nature Methods*, 13(12),
1100 1050–1054. <https://doi.org/10.1038/nmeth.4035>

1101 Choudhury, F. K., Rivero, R. M., Blumwald, E., & Mittler, R. (2017). Reactive oxygen species,
1102 abiotic stress and stress combination. *The Plant Journal*, 90(5), 856–867.
1103 <https://doi.org/10.1111/tpj.13299>

1104 Cleavitt, N. L., Battles, J. J., Johnson, C. E., & Fahey, T. J. (2017). Long-term decline of sugar
1105 maple following forest harvest, Hubbard Brook Experimental Forest, New Hampshire.
1106 *Canadian Journal of Forest Research*. <https://doi.org/10.1139/cjfr-2017-0233>

1107 Conner, J., & Liu, Z. (2000). LEUNIG, a putative transcriptional corepressor that regulates
1108 AGAMOUS expression during flower development. *Proceedings of the National*
1109 *Academy of Sciences*, 97(23), 12902–12907. <https://doi.org/10.1073/pnas.230352397>

1110 Contreras, R. N., & Shearer, K. (2018). Genome Size, Ploidy, and Base Composition of Wild and
1111 Cultivated Acer. *Journal of the American Society for Horticultural Science*, 143(6),
1112 470–485. <https://doi.org/10.21273/JASHS04541-18>

1113 Crowley, D., Barstow, M., Rivers, M., & Harvey-Brown, Y. (2020). *The Red List of Acer*. BGCI.

1114 Daspute, A. A., Sadhukhan, A., Tokizawa, M., Kobayashi, Y., Panda, S. K., & Koyama, H.
1115 (2017). Transcriptional Regulation of Aluminum-Tolerance Genes in Higher Plants:
1116 Clarifying the Underlying Molecular Mechanisms. *Frontiers in Plant Science*, 8.
1117 <https://doi.org/10.3389/fpls.2017.01358>

1118 Dawson, T. E., & Ehleringer, J. R. (1993). Gender-Specific Physiology, Carbon Isotope

- 1119 Discrimination, and Habitat Distribution in Boxelder, *Acer Negundo*. *Ecology*, 74(3),
1120 798–815. <https://doi.org/10.2307/1940807>
- 1121 De Angeli, A., Zhang, J., Meyer, S., & Martinoia, E. (2013). AtALMT9 is a malate-activated
1122 vacuolar chloride channel required for stomatal opening in Arabidopsis. *Nature*
1123 *Communications*, 4(1), 1804. <https://doi.org/10.1038/ncomms2815>
- 1124 Ding, X., Cao, Y., Huang, L., Zhao, J., Xu, C., Li, X., & Wang, S. (2008). Activation of the
1125 Indole-3-Acetic Acid–Amido Synthetase GH3-8 Suppresses Expansin Expression and
1126 Promotes Salicylate- and Jasmonate-Independent Basal Immunity in Rice. *The Plant*
1127 *Cell*, 20(1), 228–240. <https://doi.org/10.1105/tpc.107.055657>
- 1128 Donaldson, J. G., & Jackson, C. L. (2011). ARF family G proteins and their regulators: Roles in
1129 membrane transport, development and disease. *Nature Reviews. Molecular Cell Biology*,
1130 12(6), 362–375. <https://doi.org/10.1038/nrm3117>
- 1131 Du, Q., Wang, L., Zhou, D., Yang, H., Gong, C., Pan, W., & Zhang, D. (2014). Allelic variation
1132 within the S-adenosyl-L-homocysteine hydrolase gene family is associated with wood
1133 properties in Chinese white poplar (*Populus tomentosa*). *BMC Genetics*, 15(1), S4.
1134 <https://doi.org/10.1186/1471-2156-15-S1-S4>
- 1135 Dudchenko, O., Batra, S. S., Omer, A. D., Nyquist, S. K., Hoeger, M., Durand, N. C., Shamim,
1136 M. S., Machol, I., Lander, E. S., Aiden, A. P., & Aiden, E. L. (2017). De novo assembly
1137 of the *Aedes aegypti* genome using Hi-C yields chromosome-length scaffolds. *Science*,
1138 356(6333), 92–95. <https://doi.org/10.1126/science.aal3327>
- 1139 Dudchenko, O., Shamim, M. S., Batra, S. S., Durand, N. C., Musial, N. T., Mostofa, R., Pham,
1140 M., Hilaire, B. G. S., Yao, W., Stamenova, E., Hoeger, M., Nyquist, S. K., Korchina, V.,

1141 Pletch, K., Flanagan, J. P., Tomaszewicz, A., McAloose, D., Estrada, C. P., Novak, B. J.,
 1142 ... Aiden, E. L. (2018). The Juicebox Assembly Tools module facilitates de novo
 1143 assembly of mammalian genomes with chromosome-length scaffolds for under \$1000.
 1144 *BioRxiv*, 254797. <https://doi.org/10.1101/254797>

1145 Durand, N. C., Robinson, J. T., Shamim, M. S., Machol, I., Mesirov, J. P., Lander, E. S., &
 1146 Aiden, E. L. (2016). Juicebox Provides a Visualization System for Hi-C Contact Maps
 1147 with Unlimited Zoom. *Cell Systems*, 3(1), 99–101.
 1148 <https://doi.org/10.1016/j.cels.2015.07.012>

1149 Durand, N. C., Shamim, M. S., Machol, I., Rao, S. S. P., Huntley, M. H., Lander, E. S., & Aiden,
 1150 E. L. (2016). Juicer Provides a One-Click System for Analyzing Loop-Resolution Hi-C
 1151 Experiments. *Cell Systems*, 3(1), 95–98. <https://doi.org/10.1016/j.cels.2016.07.002>

1152 Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST.
 1153 *Bioinformatics*, 26(19), 2460–2461. <https://doi.org/10.1093/bioinformatics/btq461>

1154 Emms, D. M., & Kelly, S. (2015). OrthoFinder: Solving fundamental biases in whole genome
 1155 comparisons dramatically improves orthogroup inference accuracy. *Genome Biology*,
 1156 16(1), 157. <https://doi.org/10.1186/s13059-015-0721-2>

1157 Emms, D. M., & Kelly, S. (2018). OrthoFinder2: Fast and accurate phylogenomic orthology
 1158 analysis from gene sequences. *BioRxiv*. <https://doi.org/10.1101/466201>

1159 Enquist, B., Condit, R., Peet, R., Schildhauer, M., & Thiers, B. (2016). Cyberinfrastructure for an
 1160 integrated botanical information network to investigate the ecological impacts of global
 1161 climate change on plant biodiversity. *PeerJ Preprints* 4: E2615v2. *Int. J. Climatol*, 37,
 1162 4302–4315.

1163 Estravis-Barcala, M., Mattera, M. G., Soliani, C., Bellora, N., Opgenoorth, L., Heer, K., &
1164 Arana, M. V. (2020). Molecular bases of responses to abiotic stress in trees. *Journal of*
1165 *Experimental Botany*, 71(13), 3765–3779. <https://doi.org/10.1093/jxb/erz532>
1166 Faust, G. G., & Hall, I. M. (2014). SAMBLASTER: Fast duplicate marking and structural
1167 variant read extraction. *Bioinformatics*, 30(17), 2503–2505.
1168 <https://doi.org/10.1093/bioinformatics/btu314>
1169 Flynn, J. M., Hubley, R., Goubert, C., Rosen, J., Clark, A. G., Feschotte, C., & Smit, A. F.
1170 (2020). RepeatModeler2 for automated genomic discovery of transposable element
1171 families. *Proceedings of the National Academy of Sciences*, 117(17), 9451–9457.
1172 <https://doi.org/10.1073/pnas.1921046117>
1173 Fu, X., Yang, H., Pangestu, F., & Nikolau, B. J. (2020). Failure to Maintain Acetate Homeostasis
1174 by Acetate-Activating Enzymes Impacts Plant Development. *Plant Physiology*, 182(3),
1175 1256–1271. <https://doi.org/10.1104/pp.19.01162>
1176 *Genome Assembly Cookbook*. (2019). DNA Zoo. <https://www.dnazoo.org/methods>
1177 Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., Adiconis, X.,
1178 Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A.,
1179 Rhind, N., di Palma, F., Birren, B. W., Nusbaum, C., Lindblad-Toh, K., ... Regev, A.
1180 (2011). Full-length transcriptome assembly from RNA-Seq data without a reference
1181 genome. *Nature Biotechnology*, 29(7), 644–652. <https://doi.org/10.1038/nbt.1883>
1182 Gremme, G., Brendel, V., Sparks, M. E., & Kurtz, S. (2005). Engineering a software tool for
1183 gene structure prediction in higher organisms. *Information and Software Technology*,
1184 47(15), 965–978. <https://doi.org/10.1016/j.infsof.2005.09.005>

- 1185 Halman, J. M., Schaberg, P. G., Hawley, G. J., Hansen, C. F., & Fahey, T. J. (2015). Differential
1186 impacts of calcium and aluminum treatments on sugar maple and American beech growth
1187 dynamics. *Canadian Journal of Forest Research*, 45(1), 52–59.
1188 <https://doi.org/10.1139/cjfr-2014-0250>
- 1189 Halman, J. M., Schaberg, P. G., Hawley, G. J., Pardo, L. H., & Fahey, T. J. (2013). Calcium and
1190 aluminum impacts on sugar maple physiology in a northern hardwood forest. *Tree*
1191 *Physiology*, 33(11), 1242–1251. <https://doi.org/10.1093/treephys/tpt099>
- 1192 Hart, A. J., Ginzburg, S., Xu, M. (Sam), Fisher, C. R., Rahmatpour, N., Mitton, J. B., Paul, R., &
1193 Wegrzyn, J. L. (2018). EnTAP: Bringing Faster and Smarter Functional Annotation to
1194 Non-Model Eukaryotic Transcriptomes. *BioRxiv*, 307868. <https://doi.org/10.1101/307868>
- 1195 Hart, A. J., Ginzburg, S., Xu, M. (Sam), Fisher, C. R., Rahmatpour, N., Mitton, J. B., Paul, R., &
1196 Wegrzyn, J. L. (2020). EnTAP: Bringing faster and smarter functional annotation to
1197 non-model eukaryotic transcriptomes. *Molecular Ecology Resources*, 20(2), 591–604.
1198 <https://doi.org/10.1111/1755-0998.13106>
- 1199 Hasan, M. K., Cheng, Y., Kanwar, M. K., Chu, X.-Y., Ahammed, G. J., & Qi, Z.-Y. (2017).
1200 Responses of Plant Proteins to Heavy Metal Stress—A Review. *Frontiers in Plant*
1201 *Science*, 8. <https://doi.org/10.3389/fpls.2017.01492>
- 1202 He, F., Li, H.-G., Wang, J.-J., Su, Y., Wang, H.-L., Feng, C.-H., Yang, Y., Niu, M.-X., Liu, C.,
1203 Yin, W., & Xia, X. (2019). PeSTZ1, a C2H2-type zinc finger transcription factor from
1204 Populus euphratica, enhances freezing tolerance through modulation of ROS scavenging
1205 by directly regulating PeAPX2. *Plant Biotechnology Journal*, 17(11), 2169–2183.
1206 <https://doi.org/10.1111/pbi.13130>

- 1207 He, F., Pan, Q.-H., Shi, Y., & Duan, C.-Q. (2008). Biosynthesis and Genetic Regulation of
1208 Proanthocyanidins in Plants. *Molecules*, 13(10), 2674–2703.
1209 <https://doi.org/10.3390/molecules13102674>
- 1210 Helliwell, C. A., Chandler, P. M., Poole, A., Dennis, E. S., & Peacock, W. J. (2001). The
1211 CYP88A cytochrome P450, ent-kaurenoic acid oxidase, catalyzes three steps of the
1212 gibberellin biosynthesis pathway. *Proceedings of the National Academy of Sciences*,
1213 98(4), 2065–2070. <https://doi.org/10.1073/pnas.98.4.2065>
- 1214 Hendrix, S., Keunen, E., Mertens, A. I. G., Beemster, G. T. S., Vangronsveld, J., & Cuypers, A.
1215 (2018). Cell cycle regulation in different leaves of Arabidopsis thaliana plants grown
1216 under control and cadmium-exposed conditions. *Environmental and Experimental*
1217 *Botany*, 155, 441–452. <https://doi.org/10.1016/j.envexpbot.2018.06.026>
- 1218 Higo, A., Kawashima, T., Borg, M., Zhao, M., López-Vidriero, I., Sakayama, H., Montgomery,
1219 S. A., Sekimoto, H., Hackenberg, D., Shimamura, M., Nishiyama, T., Sakakibara, K.,
1220 Tomita, Y., Togawa, T., Kunimoto, K., Osakabe, A., Suzuki, Y., Yamato, K. T., Ishizaki,
1221 K., ... Araki, T. (2018). Transcription factor DUO1 generated by neo-functionalization is
1222 associated with evolution of sperm differentiation in plants. *Nature Communications*,
1223 9(1), 5283. <https://doi.org/10.1038/s41467-018-07728-3>
- 1224 Hoekenga, O. A., Maron, L. G., Piñeros, M. A., Cançado, G. M. A., Shaff, J., Kobayashi, Y.,
1225 Ryan, P. R., Dong, B., Delhaize, E., Sasaki, T., Matsumoto, H., Yamamoto, Y., Koyama,
1226 H., & Kochian, L. V. (2006). AtALMT1, which encodes a malate transporter, is identified
1227 as one of several genes critical for aluminum tolerance in Arabidopsis. *Proceedings of the*
1228 *National Academy of Sciences*, 103(25), 9738–9743.

1229 <https://doi.org/10.1073/pnas.0602868103>

1230 Hoff, K. J., Lange, S., Lomsadze, A., Borodovsky, M., & Stanke, M. (2016). BRAKER1:
 1231 Unsupervised RNA-Seq-Based Genome Annotation with GeneMark-ET and
 1232 AUGUSTUS. *Bioinformatics*, 32(5), 767–769.
 1233 <https://doi.org/10.1093/bioinformatics/btv661>

1234 Hu, B., Wang, N., Bi, X., Karaaslan, E. S., Weber, A.-L., Zhu, W., Berendzen, K. W., & Liu, C.
 1235 (2019). Plant lamin-like proteins mediate chromatin tethering at the nuclear periphery.
 1236 *Genome Biology*, 20(1), 87. <https://doi.org/10.1186/s13059-019-1694-3>

1237 Huang, J., Zhao, X., & Chory, J. (2019). The Arabidopsis Transcriptome Responds Specifically
 1238 and Dynamically to High Light Stress. *Cell Reports*, 29(12), 4186-4199.e3.
 1239 <https://doi.org/10.1016/j.celrep.2019.11.051>

1240 Huggett, B. A., Schaberg, P. G., Hawley, G. J., & Eagar, C. (2007). Long-term calcium addition
 1241 increases growth release, wound closure, and health of sugar maple (*Acer saccharum*)
 1242 trees at the Hubbard Brook Experimental Forest. *Canadian Journal of Forest Research*,
 1243 37(9), 1692–1700. <https://doi.org/10.1139/X07-042>

1244 Jaillon, O., Aury, J.-M., Noel, B., Policriti, A., Clepet, C., Casagrande, A., Choisne, N.,
 1245 Aubourg, S., Vitulo, N., Jubin, C., Vezzi, A., Legeai, F., Hugueney, P., Dasilva, C.,
 1246 Horner, D., Mica, E., Jublot, D., Poulain, J., Bruyère, C., ... French-Italian Public
 1247 Consortium for Grapevine Genome Characterization. (2007). The grapevine genome
 1248 sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature*,
 1249 449(7161), 463–467. <https://doi.org/10.1038/nature06148>

1250 Jansen, S., Broadley, M. R., Robbrecht, E., & Smets, E. (2002). Aluminum hyperaccumulation in

1251 angiosperms: A review of its phylogenetic significance. *The Botanical Review*, 68(2),
1252 235–269. [https://doi.org/10.1663/0006-8101\(2002\)068\[0235:AHIAAR\]2.0.CO;2](https://doi.org/10.1663/0006-8101(2002)068[0235:AHIAAR]2.0.CO;2)
1253 Jasinski, S., Fabrisin, I., Masson, A., Marmagne, A., Lécureuil, A., Bill, L., & Chardon, F.
1254 (2021). ACCELERATED CELL DEATH 6 Acts on Natural Leaf Senescence and
1255 Nitrogen Fluxes in Arabidopsis. *Frontiers in Plant Science*, 11.
1256 <https://doi.org/10.3389/fpls.2020.611170>
1257 Jockusch, S., & Kräutler, B. (2020). The red chlorophyll catabolite (RCC) is an inefficient
1258 sensitizer of singlet oxygen – photochemical studies of the methyl ester of RCC.
1259 *Photochemical & Photobiological Sciences*, 19(5), 668–673.
1260 <https://doi.org/10.1039/D0PP00071J>
1261 Kar, D., Pradhan, A. A., & Datta, S. (2021). The role of solute transporters in aluminum toxicity
1262 and tolerance. *Physiologia Plantarum*, 171(4), 638–652.
1263 <https://doi.org/10.1111/ppl.13214>
1264 Kidokoro, S., Yoneda, K., Takasaki, H., Takahashi, F., Shinozaki, K., & Yamaguchi-Shinozaki,
1265 K. (2017). Different Cold-Signaling Pathways Function in the Responses to Rapid and
1266 Gradual Decreases in Temperature. *The Plant Cell*, 29(4), 760–774.
1267 <https://doi.org/10.1105/tpc.16.00669>
1268 Kim, D., Langmead, B., & Salzberg, S. L. (2015). HISAT: A fast spliced aligner with low
1269 memory requirements. *Nature Methods*, 12(4), 357–360.
1270 <https://doi.org/10.1038/nmeth.3317>
1271 Kolmogorov, M. (2019). *Fast and accurate de novo assembler for single molecule sequencing*
1272 *reads: Fenderglass/Flye* [C++]. <https://github.com/fenderglass/Flye> (Original work

published 2016)

Koren, S., Walenz, B. P., Berlin, K., Miller, J. R., Bergman, N. H., & Phillippy, A. M. (2017). Canu: Scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. *Genome Research*, 27(5), 722–736. <https://doi.org/10.1101/gr.215087.116>

Krizek, B. A. (2015). AINTEGUMENTA-LIKE genes have partly overlapping functions with AINTEGUMENTA but make distinct contributions to Arabidopsis thaliana flower development. *Journal of Experimental Botany*, 66(15), 4537–4549. <https://doi.org/10.1093/jxb/erv224>

Kunkel, T. A. (2000). DNA replication fidelity. *Annu. Rev. Biochem*, 497–529.

Lamarque, L. J., Lortie, C. J., Porté, A. J., & Delzon, S. (2015). Genetic differentiation and phenotypic plasticity in life-history traits between native and introduced populations of invasive maple trees. *Biological Invasions*, 17(4), 1109–1122. <https://doi.org/10.1007/s10530-014-0781-3>

Lamarque, L. J., Porté, A. J., Eymeric, C., Lasnier, J.-B., Lortie, C. J., & Delzon, S. (2013). A Test for Pre-Adapted Phenotypic Plasticity in the Invasive Tree *Acer negundo* L. *PLOS ONE*, 8(9), e74239. <https://doi.org/10.1371/journal.pone.0074239>

Lan, T., You, J., Kong, L., Yu, M., Liu, M., & Yang, Z. (2016). The interaction of salicylic acid and Ca²⁺ alleviates aluminum toxicity in soybean (*Glycine max* L.). *Plant Physiology and Biochemistry*, 98, 146–154. <https://doi.org/10.1016/j.plaphy.2015.11.019>

Leitch, I. J., Johnston, E., Pellicer, J., Hidalgo, O., & Bennett, M. (2019). *Angiosperm DNA C-values Database (release 9.0, Apr 2019)*. <https://cvalues.science.kew.org/search>

Li, H. (2018). Minimap2: Pairwise alignment for nucleotide sequences. *Bioinformatics*, 34(18),

1295 3094–3100. <https://doi.org/10.1093/bioinformatics/bty191>

1296 Li, J., Stukel, M., Bussies, P., Skinner, K., Lemmon, A. R., Lemmon, E. M., Brown, K.,
1297 Bekmetjev, A., & Swenson, N. G. (2019). Maple phylogeny and biogeography inferred
1298 from phylogenomic data. *Journal of Systematics and Evolution*, 57(6), 594–606.
1299 <https://doi.org/10.1111/jse.12535>

1300 Li, L.-S., Ying, J., Li, E., Ma, T., Li, M., Gong, L.-M., Wei, G., Zhang, Y., & Li, S. (2021).
1301 Arabidopsis CBP60b is a central transcriptional activator of immunity. *Plant Physiology*,
1302 *kiab164*. <https://doi.org/10.1093/plphys/kiab164>

1303 Li, N., Meng, H., Xing, H., Liang, L., Zhao, X., & Luo, K. (2017). Genome-wide analysis of
1304 MATE transporters and molecular characterization of aluminum resistance in *Populus*.
1305 *Journal of Experimental Botany*, 68(20), 5669–5683. <https://doi.org/10.1093/jxb/erx370>

1306 Li, Q., Yu, H., Cao, P. B., Fawal, N., Mathé, C., Azar, S., Cassan-Wang, H., Myburg, A. A.,
1307 Grima-Pettenati, J., Marque, C., Teulière, C., & Dunand, C. (2015). Explosive Tandem
1308 and Segmental Duplications of Multigenic Families in *Eucalyptus grandis*. *Genome*
1309 *Biology and Evolution*, 7(4), 1068–1081. <https://doi.org/10.1093/gbe/evv048>

1310 Li, X., Mao, X., Xu, Y., Li, Y., Zhao, N., Yao, J., Dong, Y., Tigabu, M., Zhao, X., & Li, S.
1311 (2021). Comparative transcriptomic analysis reveals the coordinated mechanisms of
1312 *Populus × canadensis* ‘Neva’ leaves in response to cadmium stress. *Ecotoxicology and*
1313 *Environmental Safety*, 216, 112179. <https://doi.org/10.1016/j.ecoenv.2021.112179>

1314 Liang, S. C., Hartwig, B., Perera, P., Mora-García, S., Leau, E. de, Thornton, H., Alves, F. L. de,
1315 Rapsilber, J., Yang, S., James, G. V., Schneeberger, K., Finnegan, E. J., Turck, F., &
1316 Goodrich, J. (2015). Kicking against the PRCs – A Domesticated Transposase

1317 Antagonises Silencing Mediated by Polycomb Group Proteins and Is an Accessory
1318 Component of Polycomb Repressive Complex 2. *PLOS Genetics*, 11(12), e1005660.
1319 <https://doi.org/10.1371/journal.pgen.1005660>

1320 Likens, G. E., Driscoll, C. T., Buso, D. C., Siccama, T. G., Johnson, C. E., Lovett, G. M., Fahey,
1321 T. J., Reiners, W. A., Ryan, D. F., Martin, C. W., & Bailey, S. W. (1998). The
1322 biogeochemistry of calcium at Hubbard Brook. *Biogeochemistry*, 41(2), 89–173.
1323 <https://doi.org/10.1023/A:1005984620681>

1324 Likens, G. E., & Lambert, K. F. (1998). The Importance of Long-Term Data in Addressing
1325 Regional Environmental Issues. *Northeastern Naturalist*, 5(2), 127–136.
1326 <https://doi.org/10.2307/3858583>

1327 Liu, J., Magalhaes, J. V., Shaff, J., & Kochian, L. V. (2009). Aluminum-activated citrate and
1328 malate transporters from the MATE and ALMT families function independently to confer
1329 Arabidopsis aluminum tolerance. *The Plant Journal*, 57(3), 389–399.
1330 <https://doi.org/10.1111/j.1365-313X.2008.03696.x>

1331 Liu, J., & Zhou, M. (2018). The ALMT Gene Family Performs Multiple Functions in Plants.
1332 *Agronomy*, 8(2), 20. <https://doi.org/10.3390/agronomy8020020>

1333 Lomsadze, A., Burns, P. D., & Borodovsky, M. (2014). Integration of mapped RNA-Seq reads
1334 into automatic training of eukaryotic gene finding algorithm. *Nucleic Acids Research*,
1335 42(15), e119–e119. <https://doi.org/10.1093/nar/gku557>

1336 Long, R. P., Horsley, S. B., Bailey, S. W., Hallett, R. A., & Hall, T. J. (2019). Sugar maple
1337 decline and lessons learned about Allegheny Plateau soils and landscapes. In: Stout,
1338 Susan L., Ed. *SILVAH: 50 Years of Science-Management Cooperation. Proceedings of the*

1339 *Allegheny Society of American Foresters Training Session; 2017 Sept. 20-22; Clarion,*
1340 *PA. Gen. Tech. Rep. NRS-P-186. Newtown Square, PA: U.S. Department of Agriculture,*
1341 *Forest Service, Northern Research Station: 80-97., 80–97.*
1342 <https://doi.org/10.2737/NRS-GTR-P-186-Paper8>

1343 Love, M., Anders, S., & Huber, W. (2014). Differential analysis of count data—the DESeq2
1344 package. *Genome Biol*, 15(550), 10–1186.

1345 Luo, J., Zhou, J., Li, H., Shi, W., Polle, A., Lu, M., Sun, X., & Luo, Z.-B. (2015). Global poplar
1346 root and leaf transcriptomes reveal links between growth and stress responses under
1347 nitrogen starvation and excess. *Tree Physiology*, 35(12), 1283–1302.
1348 <https://doi.org/10.1093/treephys/tpv091>

1349 Ma, Q., Sun, T., Li, S., Wen, J., Zhu, L., Yin, T., Yan, K., Xu, X., Li, S., Mao, J., Wang, Y., Jin,
1350 S., Zhao, X., & Li, Q. (2020). The *Acer truncatum* genome provides insights into
1351 nervonic acid biosynthesis. *The Plant Journal*, 104(3), 662–678.
1352 <https://doi.org/10.1111/tpj.14954>

1353 Mach, J. M., Castillo, A. R., Hoogstraten, R., & Greenberg, J. T. (2001). The
1354 Arabidopsis-accelerated cell death gene ACD2 encodes red chlorophyll catabolite
1355 reductase and suppresses the spread of disease symptoms. *Proceedings of the National*
1356 *Academy of Sciences*, 98(2), 771–776. <https://doi.org/10.1073/pnas.98.2.771>

1357 Maitner, B. S., Boyle, B., Casler, N., Condit, R., Donoghue, J., Durán, S. M., Guaderrama, D.,
1358 Hinchliff, C. E., Jørgensen, P. M., Kraft, N. J. B., McGill, B., Merow, C.,
1359 Morueta-Holme, N., Peet, R. K., Sandel, B., Schildhauer, M., Smith, S. A., Svenning,
1360 J.-C., Thiers, B., ... Enquist, B. J. (2018). The bien r package: A tool to access the

1361 Botanical Information and Ecology Network (BIEN) database. *Methods in Ecology and*
1362 *Evolution*, 9(2), 373–379. <https://doi.org/10.1111/2041-210X.12861>

1363 Mano, J., Belles-Boix, E., Babiychuk, E., Inzé, D., Torii, Y., Hiraoka, E., Takimoto, K., Slooten,
1364 L., Asada, K., & Kushnir, S. (2005). Protection against Photooxidative Injury of Tobacco
1365 Leaves by 2-Alkenal Reductase. Detoxication of Lipid Peroxide-Derived Reactive
1366 Carbonyls. *Plant Physiology*, 139(4), 1773–1783. <https://doi.org/10.1104/pp.105.070391>

1367 Mekawy, A. M. M., Assaha, D. V. M., Munehiro, R., Kohnishi, E., Nagaoka, T., Ueda, A., &
1368 Saneoka, H. (2018). Characterization of type 3 metallothionein-like gene (OsMT-3a)
1369 from rice, revealed its ability to confer tolerance to salinity and heavy metal stresses.
1370 *Environmental and Experimental Botany*, 147, 157–166.
1371 <https://doi.org/10.1016/j.envexpbot.2017.12.002>

1372 Mendes, F. K., Vanderpool, D., Fulton, B., & Hahn, M. W. (2020). CAFE 5 models variation in
1373 evolutionary rates among gene families. *Bioinformatics*, btaa1022.
1374 <https://doi.org/10.1093/bioinformatics/btaa1022>

1375 Ming, R., Hou, S., Feng, Y., Yu, Q., Dionne-Laporte, A., Saw, J. H., Senin, P., Wang, W., Ly, B.
1376 V., Lewis, K. L. T., Salzberg, S. L., Feng, L., Jones, M. R., Skelton, R. L., Murray, J. E.,
1377 Chen, C., Qian, W., Shen, J., Du, P., ... Alam, M. (2008). The draft genome of the
1378 transgenic tropical fruit tree papaya (*Carica papaya* Linnaeus). *Nature*, 452(7190),
1379 991–996. <https://doi.org/10.1038/nature06856>

1380 Mohamed, H. K., Pathak, S., Roy, D. N., Hutchinson, T. C., McLaughlin, D. L., & Kinch, J. C.
1381 (1997). RELATIONSHIP BETWEEN SUGAR MAPLE DECLINE AND
1382 CORRESPONDING CHEMICAL CHANGES IN THE STEM TISSUE. *Water, Air, and*

- 1383 *Soil Pollution*, 96(1), 321–327. <https://doi.org/10.1023/A:1026429122530>
- 1384 Moore, J.-D., & Ouimet, R. (2021). Liming still positively influences sugar maple nutrition,
1385 vigor and growth, 20 years after a single application. *Forest Ecology and Management*,
1386 490, 119103. <https://doi.org/10.1016/j.foreco.2021.119103>
- 1387 Naslavsky, N., & Caplan, S. (2005). C-terminal EH-domain-containing proteins: Consensus for a
1388 role in endocytic trafficking, EH? *Journal of Cell Science*, 118(18), 4093–4101.
1389 <https://doi.org/10.1242/jcs.02595>
- 1390 Nisa, M.-U., Huang, Y., Benhamed, M., & Raynaud, C. (2019). The Plant DNA Damage
1391 Response: Signaling Pathways Leading to Growth Inhibition and Putative Role in
1392 Response to Stress Conditions. *Frontiers in Plant Science*, 10.
1393 <https://doi.org/10.3389/fpls.2019.00653>
- 1394 Nkongolo, K., Theriault, G., & Michael, P. (2018). Nickel-induced global gene expressions in
1395 red maple (*Acer rubrum*): Effect of nickel concentrations. *Plant Gene*, 14, 29–36.
1396 <https://doi.org/10.1016/j.plgene.2018.04.003>
- 1397 Nowell, R. W., Almeida, P., Wilson, C. G., Smith, T. P., Fontaneto, D., Crisp, A., Micklem, G.,
1398 Tunnacliffe, A., Boschetti, C., & Barraclough, T. G. (2018). Comparative genomics of
1399 bdelloid rotifers: Insights from desiccating and nondesiccating species. *PLOS Biology*,
1400 16(4), e2004830. <https://doi.org/10.1371/journal.pbio.2004830>
- 1401 Pellicer, J., Hidalgo, O., Dodsworth, S., & Leitch, I. J. (2018). Genome Size Diversity and Its
1402 Impact on the Evolution of Land Plants. *Genes*, 9(2), 88.
1403 <https://doi.org/10.3390/genes9020088>
- 1404 Pereira, J. F., & Ryan, P. R. (2019). The role of transposable elements in the evolution of

1405 aluminium resistance in plants. *Journal of Experimental Botany*, 70(1), 41–54.

1406 <https://doi.org/10.1093/jxb/ery357>

1407 Perteu, G. (2018). *GffCompare*. <http://ccb.jhu.edu/software/stringtie/gffcompare.shtml>

1408 Porté, A. J., Lamarque, L. J., Lortie, C. J., Michalet, R., & Delzon, S. (2011). Invasive Acer

1409 negundo outperforms native species in non-limiting resource environments due to its

1410 higher phenotypic plasticity. *BMC Ecology*, 11(1), 28.

1411 <https://doi.org/10.1186/1472-6785-11-28>

1412 Pyšek, P., Skálová, H., Čuda, J., Guo, W.-Y., Suda, J., Doležal, J., Kauzál, O., Lambertini, C.,

1413 Lučanová, M., Mandáková, T., Moravcová, L., Pyšková, K., Brix, H., & Meyerson, L. A.

1414 (2018). Small genome separates native and invasive populations in an ecologically

1415 important cosmopolitan grass. *Ecology*, 99(1), 79–90. <https://doi.org/10.1002/ecy.2068>

1416 Qiao, X., Li, Q., Yin, H., Qi, K., Li, L., Wang, R., Zhang, S., & Paterson, A. H. (2019). Gene

1417 duplication and evolution in recurring polyploidization–diploidization cycles in plants.

1418 *Genome Biology*, 20(1), 38. <https://doi.org/10.1186/s13059-019-1650-2>

1419 Racero, M., & J, F. (2020). *Caracterización funcional del canal aniónico ALMT10*.

1420 <https://digital.csic.es/handle/10261/229928>

1421 Raudvere, U., Kolberg, L., Kuzmin, I., Arak, T., Adler, P., Peterson, H., & Vilo, J. (2019).

1422 g:Profiler: A web server for functional enrichment analysis and conversions of gene lists

1423 (2019 update). *Nucleic Acids Research*, 47(W1), W191–W198.

1424 <https://doi.org/10.1093/nar/gkz369>

1425 Renner, S. S., Beenken, L., Grimm, G. W., Kocyan, A., & Ricklefs, R. E. (2007). The Evolution

1426 of Dioecy, Heterodichogamy, and Labile Sex Expression in Acer. *Evolution*, 61(11),

1427 2701–2719. <https://doi.org/10.1111/j.1558-5646.2007.00221.x>

1428 Rizza, A., Boccaccini, A., Lopez–Vidriero, I., Costantino, P., & Vittorioso, P. (2011).
1429 Inactivation of the ELIP1 and ELIP2 genes affects Arabidopsis seed germination. *New*
1430 *Phytologist*, 190(4), 896–905. <https://doi.org/10.1111/j.1469-8137.2010.03637.x>

1431 Roach, M. J., Schmidt, S. A., & Borneman, A. R. (2018). Purge Haplotigs: Allelic contig
1432 reassignment for third-gen diploid genome assemblies. *BMC Bioinformatics*, 19(1), 460.
1433 <https://doi.org/10.1186/s12859-018-2485-7>

1434 Robineau, T., Batard, Y., Nedelkina, S., Cabello-Hurtado, F., LeRet, M., Sorokine, O.,
1435 Didierjean, L., & Werck-Reichhart, D. (1998). The Chemically Inducible Plant
1436 Cytochrome P450 CYP76B1 Actively Metabolizes Phenylureas and Other Xenobiotics1.
1437 *Plant Physiology*, 118(3), 1049–1056. <https://doi.org/10.1104/pp.118.3.1049>

1438 Roddy, A. B., Thérout-Rancourt, G., Abbo, T., Benedetti, J. W., Brodersen, C. R., Castro, M.,
1439 Castro, S., Gilbride, A. B., Jensen, B., Jiang, G.-F., Perkins, J. A., Perkins, S. D.,
1440 Loureiro, J., Syed, Z., Thompson, R. A., Kuebbing, S. E., & Simonin, K. A. (2019). The
1441 Scaling of Genome Size and Cell Size Limits Maximum Rates of Photosynthesis with
1442 Implications for Ecological Strategies. *International Journal of Plant Sciences*, 181(1),
1443 75–87. <https://doi.org/10.1086/706186>

1444 Rosario, L. C. (1988). *Acer negundo*. Fire Effects Information System (FEIS). U.S. Department
1445 of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences
1446 Laboratory. <https://www.fs.fed.us/database/feis/plants/tree/aceneg/all.html>

1447 Sade, H., Meriga, B., Surapu, V., Gadi, J., Sunita, M. S. L., Suravajhala, P., & Kavi Kishor, P. B.
1448 (2016). Toxicity and tolerance of aluminum in plants: Tailoring plants to suit to acid soils.

1449 *BioMetals*, 29(2), 187–210. <https://doi.org/10.1007/s10534-016-9910-z>

1450 Sakamoto, A. N. (2019). Translesion Synthesis in Plants: Ultraviolet Resistance and Beyond.

1451 *Frontiers in Plant Science*, 10, 1208. <https://doi.org/10.3389/fpls.2019.01208>

1452 Schaberg, P. G., DeHayes, D. H., & Hawley, G. J. (2001). Anthropogenic Calcium Depletion: A

1453 Unique Threat to Forest Ecosystem Health? *Ecosystem Health*, 7(4), 214–228.

1454 <https://doi.org/10.1046/j.1526-0992.2001.01046.x>

1455 Schaberg, P. G., Tilley, J. W., Hawley, G. J., DeHayes, D. H., & Bailey, S. W. (2006).

1456 Associations of calcium and aluminum with the growth and health of sugar maple trees in

1457 Vermont. *Forest Ecology and Management*, 223(1), 159–169.

1458 <https://doi.org/10.1016/j.foreco.2005.10.067>

1459 Schlaich, N. L. (2007). Flavin-containing monooxygenases in plants: Looking beyond detox.

1460 *Trends in Plant Science*, 12(9), 412–418. <https://doi.org/10.1016/j.tplants.2007.08.009>

1461 Shi, X., Sun, H., Chen, Y., Pan, H., & Wang, S. (2016). Transcriptome Sequencing and

1462 Expression Analysis of Cadmium (Cd) Transport and Detoxification Related Genes in

1463 Cd-Accumulating *Salix integra*. *Frontiers in Plant Science*, 7.

1464 <https://doi.org/10.3389/fpls.2016.01577>

1465 Shu, K., Zhou, W., & Yang, W. (2018). APETALA 2-domain-containing transcription factors:

1466 Focusing on abscisic acid and gibberellins antagonism. *New Phytologist*, 217(3),

1467 977–983. <https://doi.org/10.1111/nph.14880>

1468 Šmarda, P., Bureš, P., Horová, L., Leitch, I. J., Mucina, L., Pacini, E., Tichý, L., Grulich, V., &

1469 Rotreklová, O. (2014). Ecological and evolutionary significance of genomic GC content

1470 diversity in monocots. *Proceedings of the National Academy of Sciences*, 111(39),

1471 E4096–E4102. <https://doi.org/10.1073/pnas.1321152111>

1472 Smit, A., Hubley, R., & Green, P. (2013). *RepeatMasker Open-4.0*. <http://www.repeatmasker.org>

1473 Sork, V. L., Fitz-Gibbon, S. T., Puiu, D., Crepeau, M., Gugger, P. F., Sherman, R., Stevens, K.,

1474 Langley, C. H., Pellegrini, M., & Salzberg, S. L. (2016). First Draft Assembly and

1475 Annotation of the Genome of a California Endemic Oak *Quercus lobata* Née (Fagaceae).

1476 *G3: Genes, Genomes, Genetics*, 6(11), 3485–3495.

1477 <https://doi.org/10.1534/g3.116.030411>

1478 Stafford, H. A. (1988). Proanthocyanidins and the lignin connection. *Phytochemistry*, 27(1), 1–6.

1479 [https://doi.org/10.1016/0031-9422\(88\)80583-1](https://doi.org/10.1016/0031-9422(88)80583-1)

1480 Stanke, M., Diekhans, M., Baertsch, R., & Haussler, D. (2008). Using native and syntenically

1481 mapped cDNA alignments to improve de novo gene finding. *Bioinformatics*, 24(5),

1482 637–644. <https://doi.org/10.1093/bioinformatics/btn013>

1483 Stanke, M., Schöffmann, O., Morgenstern, B., & Waack, S. (2006). Gene prediction in

1484 eukaryotes with a generalized hidden Markov model that uses hints from external

1485 sources. *BMC Bioinformatics*, 7(1), 62. <https://doi.org/10.1186/1471-2105-7-62>

1486 Staton, M., Best, T., Khodwekar, S., Owusu, S., Xu, T., Xu, Y., Jennings, T., Cronn, R.,

1487 Arumuganathan, A. K., Coggeshall, M., Gailing, O., Liang, H., Romero-Severson, J.,

1488 Schlarbaum, S., & Carlson, J. E. (2015). Preliminary Genomic Characterization of Ten

1489 Hardwood Tree Species from Multiplexed Low Coverage Whole Genome Sequencing.

1490 *PLOS ONE*, 10(12), e0145031. <https://doi.org/10.1371/journal.pone.0145031>

1491 Stevens, R., Grelon, M., Vezon, D., Oh, J., Meyer, P., Perennes, C., Domenichini, S., &

1492 Bergounioux, C. (2004). A CDC45 Homolog in Arabidopsis Is Essential for Meiosis, as

- 1493 Shown by RNA Interference–Induced Gene Silencing. *The Plant Cell*, 16(1), 99–113.
- 1494 <https://doi.org/10.1105/tpc.016865>
- 1495 Suda, J., Meyerson, L. A., Leitch, I. J., & Pyšek, P. (2015). The hidden side of plant invasions:
- 1496 The role of genome size. *New Phytologist*, 205(3), 994–1007.
- 1497 <https://doi.org/10.1111/nph.13107>
- 1498 Sullivan, T. J., Lawrence, G. B., Bailey, S. W., McDonnell, T. C., Beier, C. M., Weathers, K. C.,
- 1499 McPherson, G. T., & Bishop, D. A. (2013). Effects of Acidic Deposition and Soil
- 1500 Acidification on Sugar Maple Trees in the Adirondack Mountains, New York.
- 1501 *Environmental Science & Technology*, 47(22), 12687–12694.
- 1502 <https://doi.org/10.1021/es401864w>
- 1503 Sun, X.-L., Yu, Q.-Y., Tang, L.-L., Ji, W., Bai, X., Cai, H., Liu, X.-F., Ding, X.-D., & Zhu, Y.-M.
- 1504 (2013). GsSRK, a G-type lectin S-receptor-like serine/threonine protein kinase, is a
- 1505 positive regulator of plant tolerance to salt stress. *Journal of Plant Physiology*, 170(5),
- 1506 505–515. <https://doi.org/10.1016/j.jplph.2012.11.017>
- 1507 Tang, H., Bowers, J. E., Wang, X., Ming, R., Alam, M., & Paterson, A. H. (2008). Synteny and
- 1508 Collinearity in Plant Genomes. *Science*, 320(5875), 486–488.
- 1509 <https://doi.org/10.1126/science.1153917>
- 1510 Theriault, G., Michael, P., & Nkongolo, K. (2016). Comprehensive Transcriptome Analysis of
- 1511 Response to Nickel Stress in White Birch (*Betula papyrifera*). *PLOS ONE*, 11(4),
- 1512 e0153762. <https://doi.org/10.1371/journal.pone.0153762>
- 1513 Tian, J., & Kong, Z. (2019). The role of the augmin complex in establishing microtubule arrays.
- 1514 *Journal of Experimental Botany*, 70(12), 3035–3041. <https://doi.org/10.1093/jxb/erz123>

1515 Tokizawa, M., Kobayashi, Y., Saito, T., Kobayashi, M., Iuchi, S., Nomoto, M., Tada, Y.,
1516 Yamamoto, Y. Y., & Koyama, H. (2015). SENSITIVE TO PROTON
1517 RHIZOTOXICITY1, CALMODULIN BINDING TRANSCRIPTION ACTIVATOR2,
1518 and Other Transcription Factors Are Involved in ALUMINUM-ACTIVATED MALATE
1519 TRANSPORTER1 Expression. *Plant Physiology*, 167(3), 991–1003.
1520 <https://doi.org/10.1104/pp.114.256552>

1521 Tommasini, R., Vogt, E., Fromenteau, M., Hörtensteiner, S., Matile, P., Amrhein, N., &
1522 Martinoia, E. (1998). An ABC-transporter of *Arabidopsis thaliana* has both
1523 glutathione-conjugate and chlorophyll catabolite transport activity. *The Plant Journal*,
1524 13(6), 773–780. <https://doi.org/10.1046/j.1365-313X.1998.00076.x>

1525 Toyota, M., Spencer, D., Sawai-Toyota, S., Jiaqi, W., Zhang, T., Koo, A. J., Howe, G. A., &
1526 Gilroy, S. (2018). Glutamate triggers long-distance, calcium-based plant defense
1527 signaling. *Science*, 361(6407), 1112–1115. <https://doi.org/10.1126/science.aat7744>

1528 Trávníček, P., Čertner, M., Ponert, J., Chumová, Z., Jersáková, J., & Suda, J. (2019). Diversity in
1529 genome size and GC content shows adaptive potential in orchids and is closely linked to
1530 partial endoreplication, plant life-history traits and climatic conditions. *New Phytologist*,
1531 224(4), 1642–1656. <https://doi.org/10.1111/nph.15996>

1532 Tuskan, G. A., DiFazio, S., Jansson, S., Bohlmann, J., Grigoriev, I., Hellsten, U., Putnam, N.,
1533 Ralph, S., Rombauts, S., Salamov, A., Schein, J., Sterck, L., Aerts, A., Bhalerao, R. R.,
1534 Bhalerao, R. P., Blaudez, D., Boerjan, W., Brun, A., Brunner, A., ... Rokhsar, D. (2006).
1535 The Genome of Black Cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science*,
1536 313(5793), 1596–1604. <https://doi.org/10.1126/science.1128691>

- 1537 Van Bel, M., Bucchini, F., & Vandepoele, K. (2019). Gene space completeness in complex plant
1538 genomes. *Current Opinion in Plant Biology*, 48, 9–17.
1539 <https://doi.org/10.1016/j.pbi.2019.01.001>
- 1540 Veeckman, E., Ruttink, T., & Vandepoele, K. (2016). Are We There Yet? Reliably Estimating the
1541 Completeness of Plant Genome Sequences. *The Plant Cell*, 28(8), 1759–1768.
1542 <https://doi.org/10.1105/tpc.16.00349>
- 1543 Veleba, A., Šmarda, P., Zedek, F., Horová, L., Šmerda, J., & Bureš, P. (2017). Evolution of
1544 genome size and genomic GC content in carnivorous holokinetics (Droseraceae). *Annals*
1545 *of Botany*, 119(3), 409–416. <https://doi.org/10.1093/aob/mcw229>
- 1546 Verde, I., Jenkins, J., Dondini, L., Micali, S., Pagliarani, G., Vendramin, E., Paris, R., Aramini,
1547 V., Gazza, L., Rossini, L., Bassi, D., Troggio, M., Shu, S., Grimwood, J., Tartarini, S.,
1548 Dettori, M. T., & Schmutz, J. (2017). The Peach v2.0 release: High-resolution linkage
1549 mapping and deep resequencing improve chromosome-scale assembly and contiguity.
1550 *BMC Genomics*, 18(1), 225. <https://doi.org/10.1186/s12864-017-3606-9>
- 1551 Vurture, G. W., Sedlazeck, F. J., Nattestad, M., Underwood, C. J., Fang, H., Gurtowski, J., &
1552 Schatz, M. C. (2017). GenomeScope: Fast reference-free genome profiling from short
1553 reads. *Bioinformatics*, 33(14), 2202–2204. <https://doi.org/10.1093/bioinformatics/btx153>
- 1554 Wang, Y., Zhao, Y., Wang, S., Liu, J., Wang, X., Han, Y., & Liu, F. (2021). Up-regulated
1555 2-alkenal reductase expression improves low-nitrogen tolerance in maize by alleviating
1556 oxidative stress. *Plant, Cell & Environment*, 44(2), 559–573.
1557 <https://doi.org/10.1111/pce.13956>
- 1558 Wu, T. D., & Watanabe, C. K. (2005). GMAP: A genomic mapping and alignment program for

1559 mRNA and EST sequences. *Bioinformatics*, 21(9), 1859–1875.

1560 <https://doi.org/10.1093/bioinformatics/bti310>

1561 Wu, X., Oh, M.-H., Schwarz, E. M., Larue, C. T., Sivaguru, M., Imai, B. S., Yau, P. M., Ort, D.

1562 R., & Huber, S. C. (2011). Lysine Acetylation Is a Widespread Protein Modification for

1563 Diverse Proteins in Arabidopsis. *Plant Physiology*, 155(4), 1769–1778.

1564 <https://doi.org/10.1104/pp.110.165852>

1565 Xia, K., Ou, X., Tang, H., Wang, R., Wu, P., Jia, Y., Wei, X., Xu, X., Kang, S.-H., Kim, S.-K., &

1566 Zhang, M. (2015). Rice microRNA osa-miR1848 targets the obtusifoliol

1567 14 α -demethylase gene OsCYP51G3 and mediates the biosynthesis of phytosterols and

1568 brassinosteroids during development and in response to stress. *New Phytologist*, 208(3),

1569 790–802. <https://doi.org/10.1111/nph.13513>

1570 Xu, M., Cho, E., Burch-Smith, T. M., & Zambryski, P. C. (2012). Plasmodesmata formation and

1571 cell-to-cell transport are reduced in decreased size exclusion limit 1 during

1572 embryogenesis in Arabidopsis. *Proceedings of the National Academy of Sciences*,

1573 109(13), 5098–5103. <https://doi.org/10.1073/pnas.1202919109>

1574 Yamamoto, Y., Kobayashi, Y., Devi, S. R., Rikiishi, S., & Matsumoto, H. (2003). Oxidative

1575 stress triggered by aluminum in plant roots. In J. Abe (Ed.), *Roots: The Dynamic*

1576 *Interface between Plants and the Earth: The 6th Symposium of the International Society*

1577 *of Root Research, 11–15 November 2001, Nagoya, Japan* (pp. 239–243). Springer

1578 Netherlands. https://doi.org/10.1007/978-94-017-2923-9_23

1579 Yang, J., Wariss, H. M., Tao, L., Zhang, R., Yun, Q., Hollingsworth, P., Dao, Z., Luo, G., Guo,

1580 H., Ma, Y., & Sun, W. (2019). De novo genome assembly of the endangered Acer

1581 yangbiense, a plant species with extremely small populations endemic to Yunnan
1582 Province, China. *GigaScience*, 8(7). <https://doi.org/10.1093/gigascience/giz085>

1583 Yang, W.-Y., Zheng, Y., Bahn, S. C., Pan, X.-Q., Li, M.-Y., Vu, H. S., Roth, M. R., Scheu, B.,
1584 Welti, R., Hong, Y.-Y., & Wang, X.-M. (2012). The Patatin-Containing Phospholipase A
1585 pPLAII α Modulates Oxylipin Formation and Water Loss in *Arabidopsis thaliana*.
1586 *Molecular Plant*, 5(2), 452–460. <https://doi.org/10.1093/mp/ssr118>

1587 Yang, Z.-B., Geng, X., He, C., Zhang, F., Wang, R., Horst, W. J., & Ding, Z. (2014).
1588 TAA1-Regulated Local Auxin Biosynthesis in the Root-Apex Transition Zone Mediates
1589 the Aluminum-Induced Inhibition of Root Growth in *Arabidopsis*. *The Plant Cell*, 26(7),
1590 2889–2904. <https://doi.org/10.1105/tpc.114.127993>

1591 Zeng, L., Tu, X.-L., Dai, H., Han, F.-M., Lu, B.-S., Wang, M.-S., Nanaei, H. A., Tajabadipour,
1592 A., Mansouri, M., Li, X.-L., Ji, L.-L., Irwin, D. M., Zhou, H., Liu, M., Zheng, H.-K.,
1593 Esmailzadeh, A., & Wu, D.-D. (2019). Whole genomes and transcriptomes reveal
1594 adaptation and domestication of pistachio. *Genome Biology*, 20(1), 79.
1595 <https://doi.org/10.1186/s13059-019-1686-3>

1596 Zhang, Y., Guo, J., Chen, M., Li, L., Wang, L., & Huang, C.-F. (2018). The Cell Cycle
1597 Checkpoint Regulator ATR Is Required for Internal Aluminum Toxicity-Mediated Root
1598 Growth Inhibition in *Arabidopsis*. *Frontiers in Plant Science*, 9.
1599 <https://doi.org/10.3389/fpls.2018.00118>

1600 Zhao, D., Hamilton, J. P., Bhat, W. W., Johnson, S. R., Godden, G. T., Kinser, T. J., Boachon, B.,
1601 Dudareva, N., Soltis, D. E., Soltis, P. S., Hamberger, B., & Buell, C. R. (2019). A
1602 chromosomal-scale genome assembly of *Tectona grandis* reveals the importance of

tandem gene duplication and enables discovery of genes in natural product biosynthetic pathways. *GigaScience*, 8(3). <https://doi.org/10.1093/gigascience/giz005>

Zhu, W., Li, L., Neuhäuser, B., Thelen, M., Wang, M., Chen, J., Wei, L., Venkataramani, K., Exposito-Alonso, M., Liu, C., Keck, J., Barragan, A. C., Schwab, R., Lutz, U., Ludewig, U., & Weigel, D. (2021). Small peptides modulate the immune function of the ion channel-like protein ACD6 in *Arabidopsis thaliana*. *BioRxiv*, 2021.01.25.428077. <https://doi.org/10.1101/2021.01.25.428077>

Figure and Table Captions

Figure 1. a) Native distributions of *A. saccharum* (blue) and *A. negundo* (orange) in North America. Leaves indicate location of individuals selected for the reference genomes; *A. saccharum* from the University of Maryland campus, and *A. negundo* from the Smithsonian Environmental Research Center. HBEF (Hubbard Brook Experimental Forest) is the location of the 9 individuals used for RNA-seq. b) All records of occurrence, native, introduced, and unknown, per BIEN 4.2. Non-native occurrences are predominantly *A. negundo*. <https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/figures/aceranges.jpg>

Figure 2. Results of assembly testing with *A. saccharum*, comparing fragmentation in terms of total contigs versus assembly length. The dashed line represents the estimated genome size. Gray dots are short-read assemblers, shown as highly fragmented. Blue dots are long-read tests of assembly workflows. Canu refers to the use of reads error-corrected by the Canu pipeline. The red dot is the selected draft assembly, and the green dot shows scaffolding results following Hi-C. Detailed assembly statistics are available in [File S1](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/figures/assemblycomparisongraph_busco.jpg). https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/figures/assemblycomparisongraph_busco.jpg

1630

1631 **Figure 3.** Ks distribution for WGD synten blocks with a summary of duplication types in (a) *A.*
1632 *negundo* and (b) *A. saccharum*. Abbreviations for categories of duplication: WGD, whole
1633 genome duplication; TD, tandem duplication; PD, proximal duplication; TRD, transposed
1634 duplication; DSD, dispersed duplication. (c) Circos plot of the thirteen chromosomes ordered
1635 largest to smallest for *A. negundo* (orange bars) and *A. saccharum* (blue bars) with distributions
1636 of gene density (green) and transposable element frequency (purple). Syntenic regions are linked
1637 in gray with darker shades to visually highlight larger recombinations.
1638 [https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/figures/acs](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/figures/acs_a_acne_manuscript_circos.png)
1639 [a_acne_manuscript_circos.png](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/figures/acs_a_acne_manuscript_circos.png)

1640

1641 **Figure 4.** a) Differential expression study design showing number of samples collected in fall
1642 and spring from treatment plots at the Hubbard Brook Experimental Forest, Nutrient Perturbation
1643 study. b) Differentially expressed genes (up and downregulated) for each treatment and season
1644 comparison. Charts display both significance and relative expression denoted as log-fold change.
1645 Dotted lines indicate thresholds of significance (0.1 p-adjusted, 1.5 log₂ fold change).
1646 [https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/figures/hbe](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/figures/hbe_fplots.jpg)
1647 [fplots.jpg](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/figures/hbe_fplots.jpg)

1648

1649 **Figure 5.** a) Gene ontology enrichments for *Acer* (all three species combined), *A. negundo*, and
1650 *A. saccharum*. Abbreviations for gene family dynamics: E, expanded; N, novel; RC, rapidly
1651 contracting. b) Total gene families, shared and unique, among the *Acer*. c) Reconstructed gene
1652 tree showing contracted gene families in red and expanded in green.
1653 [https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/figures/caf](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/figures/caf_etreevennGO.jpg)
1654 [etreevennGO.jpg](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/figures/caf_etreevennGO.jpg)

1655

1656 **Figure 6.** *A. negundo* gene families with ontology related to DNA damage and repair, and
1657 secondary enrichments categorized by color. Circles with multiple colors indicate multiple

ontology assignments. Lines indicated known or predicted interactions, or other association via text-mining, co-expression, or protein homology.

https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/figures/acne_dnadamage.jpg

Figure 7. Orthogroup sizes for aluminum tolerance gene families are presented by species. Families were selected for inclusion based on documented aluminum tolerance and/or presence in the HBEF RNA-Seq differential expression results. Color represents the proportion of gene membership per species, with darker purple equating to more contracted families relative to the median, and dark green indicating expansion. (H) Family contains HBEF differentially expressed gene; (E) Expanded in *A. saccharum*; (C) Contracting; (M) Missing; (N) Novel; (*) Rapidly expanding; Categorization of tolerance is according to literature describing aluminum stress phenotypes. The undetermined category contains species where tolerance to aluminum or acidic soils has not been reported. ¹*B. pendula* is undetermined due to high variability in tolerance by genotype.

https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/figures/genefamily_al_comparisons_manuscript_customnorm.html.jpg

Supplemental

Figure S1. Genome size estimation using k-mer distribution analysis

https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/figures/Figure_1_SuppInfo.pdf

Figure S2. Hi-C plots

https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/figures/Figure_2_SuppInfo.pdf

Figure S3. PCA plot

https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/figures/Figure_3_SuppInfo.pdf

Figure S4. Syntenic comparisons between the three *Acer* genomes

https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/figures/Figure_4_SuppInfo.pdf

Table S1. Illumina, PacBio, and Hi-C sequencing data summaries

https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/tables/sequencing_tables.pdf

Table S2. HBEF table of trees

https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/tables/hbef_trees.pdf

Table S3. HBEF GO enrichment

https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/tables/hbef_functional_enrichment.pdf

Table S4. Orthogroup statistics by species

https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/tables/Orthogroup_summary_table.pdf

Table S5. OF dynamics GO enrichment

https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/tables/orthogroup_functional_enrichment.pdf

Table S6. CAFE GO enrichment

https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/tables/cafe_functional_enrichment.pdf

Table S7. HBEF Nutrient Perturbation Treatment Schedule

<https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/tables/HBEFNuPertTreatmentTable2015.pdf>

File S1. Assembly output stats

https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/assembly_statistics.xlsx

1716 **File S2.** Annotation details

1717 [https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/annot](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/annotation_statistics.xlsx)
 1718 [ation_statistics.xlsx](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/annotation_statistics.xlsx)

1719 **File S3.** Collinearity analysis of recent Ks peak of WGD frequency

1720 [https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/Acer_](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/Acer_microsynteny.xlsx)
 1721 [microsynteny.xlsx](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/Acer_microsynteny.xlsx)

1722 **File S4.** HBEF differentially expressed genes

1723 [https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/HBEF](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/HBEF_DEGs.xlsx)
 1724 [_DEGs.xlsx](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/HBEF_DEGs.xlsx)

1725 **File S5.** Orthofinder significant dynamics

1726 [https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/orthof](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/orthofinder_dynamics.xlsx)
 1727 [inder_dynamics.xlsx](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/orthofinder_dynamics.xlsx)

1728 **File S6.** CAFE significant rapid evolution

1729 [https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/cafe_r](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/cafe_rapidly_evolving.xlsx)
 1730 [apidly_evolving.xlsx](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/cafe_rapidly_evolving.xlsx)

1731 **File S7.** *A. negundo* vs *Acer* contracted or missing using longest overall gene as annotation

1732 [https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acne_](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acne_contractedmissing_longestoverall.xlsx)
 1733 [contractedmissing_longestoverall.xlsx](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acne_contractedmissing_longestoverall.xlsx)

1734 **File S8.** *A. negundo* vs *Acer* contracted or missing using longest *Acer* gene as annotation

1735 [https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acne_](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acne_contractedmissing_longestacer.xlsx)
 1736 [contractedmissing_longestacer.xlsx](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acne_contractedmissing_longestacer.xlsx)

1737 **File S9.** *A. negundo* vs *Acer* expanded or novel using longest overall gene as annotation

1738 [https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acne_](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acne_expandednovel_longestacer.xlsx)
 1739 [expandednovel_longestacer.xlsx](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acne_expandednovel_longestacer.xlsx)

1740 **File S10.** *A. negundo* vs *Acer* expanded or novel using longest *Acer* gene as annotation

1741 [https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acne_](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acne_expandednovel_longestoverall.xlsx)
 1742 [expandednovel_longestoverall.xlsx](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acne_expandednovel_longestoverall.xlsx)

1743 **File S11.** *A. saccharum* vs *Acer* contracted or missing using longest *Acer* gene as annotation

1744 [https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acsa_](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acsa_contractedmissing_longestacer.xlsx)
1745 [contractedmissing_longestacer.xlsx](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acsa_contractedmissing_longestacer.xlsx)
1746 **File S12.** *A. saccharum* vs *Acer* contracted or missing using longest overall gene as annotation
1747 [https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acsa_](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acsa_contractedmissing_longestoverall.xlsx)
1748 [contractedmissing_longestoverall.xlsx](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acsa_contractedmissing_longestoverall.xlsx)
1749 **File S13.** *A. saccharum* vs *Acer* expanded or novel using longest *Acer* gene as annotation
1750 [https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acsa_](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acsa_expandednovel_longestacer.xlsx)
1751 [expandednovel_longestacer.xlsx](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acsa_expandednovel_longestacer.xlsx)
1752 **File S14.** *A. negundo* verified missing orthogroups
1753 [https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acne_](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acne_missing_verified_string_mapping.tsv)
1754 [missing_verified_string_mapping.tsv](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acne_missing_verified_string_mapping.tsv)
1755 **File S15.** *A. saccharum* verified missing orthogroups
1756 [https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acsa_](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acsa_missing_verified_string_mapping.tsv)
1757 [missing_verified_string_mapping.tsv](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/acsa_missing_verified_string_mapping.tsv)
1758 **File S16.** Orthogroup comparisons for HBEF DEG and Al tolerance genes
1759 [https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/hbef_](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/hbef_orthogroup_Al_comparisons.xlsx)
1760 [orthogroup_Al_comparisons.xlsx](https://gitlab.com/PlantGenomicsLab/AcerGenomes/-/blob/master/acer/supplemental/files/hbef_orthogroup_Al_comparisons.xlsx)