Core Ideas:

1) North American Popcorn is composed of two distinct populations that differ genetically and phenotypically.
2) North American Yellow Pearl Popcorns contain limited genetic diversity and are highly inbred.
3) Pointed and Latin American Popcorns exhibit considerable genetic diversity and rapid linkage disequilibrium decay.
4) Kernel color does not affect nicosulfuron sensitivity and instead reflects differences between the populations.
5) Popcorn-specific candidate genes for nicosulfuron tolerance are distinct from dent corn.

Keywords:

Popcorn, Genetic Diversity, Nicosulfuron Tolerance, Plant Genetic Resources

Genetic diversity of North American popcorn germplasm and the effect of population structure on nicosulfuron response

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Abbreviations:

ALS, acetolactate synthase; BCAA, branched-chain amino acid; CIMMYT, International Maize and Wheat Improvement Center; DAPC, discriminant analysis of principal coordinates; GBS, genotyping-by-sequencing; GRIN, Germplasm Resources Information Network; GWAS, genome-wide association studies; IBS, identity by state; LD, linkage disequilibrium; MAF, minor allele frequency; MAPQ, mapping quality; PCoA, principal coordinate analysis; SNP, single nucleotide polymorphism

ABSTRACT

Popcorn is an important crop in the United States; however, genetic analyses of popcorn are limited and tend to utilize relatively few markers that cannot capture the total genomic variation. To improve the genomic resources in popcorn, a panel of 362 popcorn accessions was evaluated using 417,218 single nucleotide polymorphisms generated using a genotyping-by-sequencing approach. Using this genomic data, a model-based clustering analysis identified two populations. The first comprised North American Yellow Pearl Popcorns and several accessions of the Chilean Curagua landrace. The second, the Pointed and Latin American Popcorns, included all remaining North American (pointed and early popcorns), Latin American, and global accessions. The two populations exhibited large differences in population structure and genetic diversity. The North American Yellow Pearl Popcorns constitute a highly inbred population with limited genetic diversity compared to the Pointed and Latin American Popcorns. Additionally, phenotypic differences between the two populations were observed in kernel color and nicosulfuron sensitivity. A filtered set of SNPs was curated and used for genome-wide association studies and popcorn-specific candidate genes for nicosulfuron tolerance were identified. The genomic characterization described here can be used by breeding programs to accelerate the rate of genetic gain and incorporate genetic diversity into elite popcorn germplasm.

1 INTRODUCTION

Popcorn (*Zea mays var. everta*) is a popular snack and a valuable commercial crop in the United States. In a global market worth 10 billion USD, the United States has the highest per capita popcorn consumption of any country at over 60 quarts per year, representing a total of 17 billion quarts consumed annually (Serna-Saldivar 2022). Likewise, the United States grows more popcorn than any other nation, with approximately 90,000 hectares of popcorn planted annually, most of which are grown in Nebraska, Indiana, Ohio, and Illinois (USDA NASS, 2022).

Popcorn has been cultivated within the territory of the modern United States since pre-Columbian times, although the range of pre-Columbian popcorn was limited to the American Southwest. Popcorn landraces first reached this region by 4,200 BP (Merrill et al. 2009), likely first from a highland origin, including the landraces Palomero Toluqueño and Palomero de Jalisco, followed by a later mixing with lowland landraces, such as Chapalote and Reventador (da Fonseca et al. 2015; Wang et al. 2017). Although these landraces do pop, the popping quality is poor compared to modern lines in terms of expansion, hull fragmentation, and percentage of unpopped kernels (Vazquez-Carrillo et al. 2019; Bautista-Ramirez et al. 2020).

Despite cultivation in the American Southwest for millennia, popcorn was not widely distributed in North America until the late 18th and early 19th centuries, and even then, the amount grown was limited (Erwin 1950). During the first half of the 19th century, popcorn was typically grown in the United States as a small-scale garden crop for household use (Ziegler 2000). By the late 19th and early 20th century, the popularity of popcorn rose as open-pollinated varieties began reaching more consumers, following the first successful processors and commercial brands in the 1880s and the introduction of the mobile popcorn machine in 1893 (Smith 1999). The predominant types of popcorn grown during this time were the North American Pointed Rice

Popcorns or 'hulless' types, characterized as having a pointed upper kernel shape and frequently colored white. The first hybrid popcorn used in commercial production was generated using two selections of Japanese Hulless, a white pointed rice variety (Brunson 1937). However, since the mid-20th century, another group of popcorn has largely replaced the pointed rice type commercially – the North American Yellow Pearl Popcorns. This group has a rounded kernel shape and is predominantly yellow, although color variation has been introduced by several breeding programs (Ziegler 2000). While the North American Yellow Pearl Popcorns are the most important for commercial production in the United States, North American Pointed Rice Popcorns are still commercially grown and used in breeding programs.

Characterizing the genetic relationships and identifying variation within these distinct populations of North American popcorn is an important aspect of breeding improved popcorn. Early molecular techniques were used to assess the genetic diversity of North American Yellow Pearl Popcorns and identified three heterotic groups – Supergold, South American, and Amber Pearl (Kantety et al. 1995). Later work by Santacruz-Varela et al. defined three major groups of North American popcorn – the aforementioned North American Pointed Rice Popcorn and North American Yellow Pearl Popcorn, as well as the North American Early Popcorn (2004). Additionally, each group's phylogenetic relationship and historical origin were investigated, and possible germplasm sources were identified. The North American Pointed Rice Popcorns appeared to originate from the traditional pointed rice popcorns of Latin America, based on both morphological and genotypic similarities. Furthermore, the North American Yellow Pearl Popcorns were most closely related to, and likely principally derived from, the Chilean Curagua landrace. Finally, the North American Early Popcorns may have been developed from Northern Flint corn and subsequently influenced by European popcorn varieties (Santacruz-Varela et al.

2004). Although this information has been useful in understanding breeding history and relatedness, the limited number of markers and lines has restricted its use in modern breeding programs to heterotic pool development.

Compared with dent corn germplasm, popcorn exhibits considerably inferior agronomic traits, including herbicide tolerance, disease resistance, lodging, yield, and overall vigor (Ziegler 2000). Additionally, differences in herbicide tolerance have been reported within popcorn, with white popcorn generally exhibiting greater herbicide sensitivity than yellow (Loux et al. 2017). Historically, the poorer agronomic traits of popcorn are due partly to the amount of time and resources breeders have invested relative to dent corn and also because of the emphasis placed on popping quality traits. In addition to agronomic traits, popcorn breeders must consider quality traits directly related to popping and eating quality, including popping expansion, hull separation, frequency of unpopped kernels, texture, and appearance (Matz 1984). Furthermore, dent corn breeding pipelines have integrated molecular characterization and resources, resulting in improved selection and rates of genetic gain, while publicly available resources within popcorn are limited.

High-quality genetic and genomic resources in maize have empowered researchers and facilitated discoveries involving the genetic architecture of traits, candidate gene association and identification, and population structure (Buckler et al. 2009; Cook et al. 2012; Romay et al. 2013; Peiffer et al. 2014; Wallace et al. 2014; Gage et al. 2020). While the genetic resources for mapping populations, diversity panels, and seed banks have been used extensively within dent corn, popcorn germplasm comprises a genetically distinct subpopulation of maize that is underrepresented in these studies. Recently, genotyping-by-sequencing (GBS) was performed on popcorn accessions from CIMMYT (Li et al. 2019; Li et al. 2021) and Chinese breeding

117 programs (Yu et al. 2021) and used to characterize genetic diversity, describe population
118 structure, and perform genome-wide association studies (GWAS). The genomic characterization
119 of these diverse popcorn accessions can be used to assist breeders in incorporating genetic
120 variation into breeding programs and identifying loci controlling important traits in popcorn.
121 Likewise, the rate of genetic gain in North American popcorn breeding programs would increase
122 dramatically with the incorporation of high-quality genetic and genomic information, as has been
123 utilized in dent corn breeding for decades.
124 In this study, molecular analysis was performed on 362 publicly available popcorn accessions
125 genotyped with GBS representing the diversity of North American popcorn. The goals of this
126 study were to i) characterize the genetic diversity of North American popcorn, ii) identify
127 population structure within North American popcorn and other groups of popcorn, iii) describe
128 genomic differences between these populations, iv) perform genome-wide association studies,
129 and v) compare findings with previous attempts to characterize North American popcorn.

130 **2 MATERIALS AND METHODS**

131 **2.1 Plant materials and DNA extraction**

132 Of publicly available lines of popcorn, 341 unique accessions were selected from the Germplasm
133 Resources Information Network (GRIN), and 21 accessions were selected from the International
134 Maize and Wheat Improvement Center (CIMMYT). Of these, 79 accessions had available
135 sequence information (Romay et al. 2013). Thus, 283 new popcorn lines were sequenced, with
136 68 selected for replicate sequencing. Additionally, four dent inbreds with available sequence
137 information were included as outgroup accessions: B73, Mo17, PH207, and Oh43.
138 Samples were prepared for DNA extraction by bulking tissue from five seedlings of each
139 accession in a 2 mL tube, collected from the youngest leaf of each approximately ten days after
140 germination. DNA was extracted from each sample using a modified CTAB DNA extraction
141 protocol and resuspended in water. DNA was quantified in 96-well plates using PicoGreen
142 (Invitrogen, Carlsbad, CA), and DNA concentrations were normalized to 50ng/µL by diluting
143 with 10 mM Tris, then stored at 4°C.

144 **2.2 Genotyping-by-sequencing library construction**

145 Genotyping-by-sequencing libraries were constructed using the protocol described by Elshire *et*
146 *al.* (2011). Briefly, restriction digestion and ligation were performed in 96-well plates using
147 *Ape*KI by adding 250 ng of DNA from each sample, 1.5µL of 0.1 µM rare barcoded adapter, and
148 0.5µL of 10 µM common barcoded adapter per well. Libraries were pooled, size-selected and
149 cleaned with Agencourt AMPure XP beads (Beckman Coulter Inc., Brea, CA), and amplified for
150 15 cycles using KAPA HiFi HotStart ReadyMix (KAPA BIOSYSTEMS). Following a final
151 purification with Agencourt AMPure XP beads, libraries were quantified using PicoGreen. A 1
152 ng/µL dilution of the library was made using 10 mM Tris, and 5 µL was run on a 1% agarose gel

153 to verify size selection and primer-dimer removal. Fragment size was estimated using a
154 Bioanalyzer 2100 (Agilent, Santa Clara, CA) High Sensitivity DNA Analysis. Pooled libraries
155 were diluted to 10 nmol using 10 mM Tris and sequenced with 100-bp, single-end reads on an
156 Illumina NovaSeq 6000 (Illumina, San Diego, CA). The sequencing data has been submitted to
157 NCBI SRA (PRJNA911304).

158 **2.3 Marker calling**

159 The reads from the 79 popcorn accessions previously sequenced by Romay et al. (2013) were
160 downloaded from NCBI SRA (SRP021921) and processed with the new raw sequencing reads
161 using the GBS v2 discovery pipeline from TASSEL 5 (release 5.2.59) (Glaubitz et al. 2014). The
162 pipeline tools and select parameters were run in the following order: First,
163 GBSSeqToTagDBPlugin, with ApeKI and a kmer length of 64, followed by
164 TagExportToFastqPlugin. Next, two alignment indices were built with Bowtie2 (Langmead &
165 Salzberg 2012) using the B73 v5 and HP301 v1 reference genomes (Hufford et al. 2021). Both
166 genome indices and output files were processed using the same parameters afterward. The
167 Bowtie2 alignment command was run using the --very-sensitive preset, resulting in an overall
168 alignment of 90.6% and 91.2% for the B73 v5 and HP301 v1 indices, respectively. The SAM
169 files were then sorted using samtools (Li et al. 2009), and mapping quality (MAPQ) scores were
170 analyzed using Qualimap 2 (Okonechnikov et al. 2016). The SAM files were read using the
171 SAMToGBSdbPlugin with a minimum MAPQ score of 20, after which the
172 DiscoverySNPCallPluginV2 was run using a minimum minor allele frequency of 0.05. Finally,
173 the ProductionSNPCallerPluginV2 was run using the same parameters as the
174 GBSSeqToTagDBPlugin.

175 **2.4 Marker coverage and characterization**

176 The genomic distribution of single nucleotide polymorphisms (SNPs) was based on the HP301
177 v1 reference genome, as the popcorn reads alignments were slightly better than when using the
178 B73 v5 genome. Markers were reported from TASSEL 5 for the Core Analysis set and each
179 chromosome. An additional marker set was generated for imputation and GWAS after filtering
180 out sites with more than 0.75 missing data from the Core Analysis set. Nucleotide substitutions
181 were calculated from the Core Analysis set using the TASSEL 5 GenotypeSummaryPlugin.
182 Markers were identified as intragenic by searching their positions relative to the start and stop
183 positions of the HP301 v1 gene models. The density and distribution of polymorphic SNPs were
184 determined in *R* (R Core Team, 2021) using the *R* package *rMVP* (Yin et al. 2021).

185 **2.5 Population structure**

186 A kinship matrix of centered identity by state (IBS) values was calculated for all possible
187 pairwise taxa comparisons in TASSEL. A plot of each pairwise comparison was generated using
188 the *R* package *ggplot2* (Wickham 2016). To formally test for population structure using
189 molecular data, an unsupervised, model-based clustering analysis was performed using
190 ADMIXTURE (Alexander et al. 2009). One through five *K* populations were tested using tenfold
191 cross-validation, with errors minimized at *K* equals two. A second ADMIXTURE analysis was
192 performed in validation after combining the 362 popcorn accessions with 2,284 additional
193 accessions sequenced from the Ames Diversity Panel (Romay et al. 2013). Due to computational
194 restraints, accessions designated stiff stalk or non-stiff stalk were excluded. Reads from the
195 combined set of accessions were aligned to the HP301 v1 reference genome using the TASSEL 5
196 GBS v2 discovery pipeline without changing any of the aforementioned parameters. In
197 ADMIXTURE, one through twenty *K* populations were tested using fivefold cross-validation,
198 with errors minimized at *K* equals fifteen.

199 Principal coordinates were generated in TASSEL from the IBS kinship matrix. Three of the
200 coordinates from the matrix with high eigenvalues were visualized using *ggplot2*. After
201 removing 30 accessions with missing data greater than 0.95, phylogenetic analysis was
202 performed using the *R* package *ape* (Paradis et al. 2004) using the neighbor-joining method from
203 a modified Euclidean distance matrix and visualized with the *R* package *ggtree* (Yu et al. 2017).
204 The neighbor-joining algorithm was selected because of the considerable differences in selection
205 and breeding history of the populations included in this study, making alternate methods such as
206 UPGMA untenable. To further investigate the relationship between teosinte and the Pointed and
207 Latin American Popcorn population, an additional neighbor-joining tree was generated. A
208 distance matrix was produced from the seventeen teosinte accessions and ten randomly selected
209 individuals from each of the fifteen ADMIXTURE groups identified from the combined popcorn
210 – Ames Diversity Panel analysis. This distance matrix was used by *ape* to create the second tree
211 and also visualized with *ggtree*.

212 **2.6 Population genetics and diversity**

213 To evaluate differences in selection and genetic drift between the two populations, the proportion
214 of monomorphic SNPs within each population was calculated. Both populations were filtered
215 from the Core Analysis set, after which markers in each population were filtered to contain a
216 minimum of two observations. Markers without minor alleles were then filtered out, allowing the
217 number of monomorphic markers to be calculated by subtracting the remaining polymorphic
218 markers. After filtering monomorphic sites, marker minor allele frequencies were calculated
219 from the Core Analysis set using the TASSEL 5 GenotypeSummaryPlugin. The distribution of
220 minor allele frequencies was calculated for each popcorn population.

221 A marker subset was produced for linkage disequilibrium (LD) analysis by removing SNPs with
222 more than 50% missing data, a MAF less than 0.05, and heterozygosity greater than 0, resulting
223 in 13,031 retained markers. Within each population, LD was calculated between the subset of
224 markers on each chromosome using the TASSEL 5 LinkageDisequilibriumPlugin. The level of
225 LD decay was measured using the squared Pearson correlation coefficient (r^2) between each pair
226 of markers. Pairwise comparisons were binned according to distance using logarithmically
227 increasing bins.

228 **2.7 Herbicide treatment**

229 A subset of popcorn accessions was evaluated for response to nicosulfuron at the University of
230 Illinois Crop Sciences Research and Education Center, Urbana, Illinois, during the summer of
231 2022. A total of 294 genotypes were included in the trial, with 111 North American Yellow Pearl
232 Popcorns and 178 Pointed and Latin American Popcorns. Additionally, five controls were
233 included in the panel – a tolerant sweet corn hybrid (GSS1477), a nicosulfuron-sensitive sweet
234 corn hybrid (Merit), a partially sensitive yellow popcorn hybrid (R400), a tolerant yellow
235 popcorn hybrid (R502), and a tolerant white popcorn hybrid (R98114W). Genotypes were
236 arranged in a randomized complete block design with three replications. An experimental unit
237 comprised a single 3-meter row containing 10-20 plants.
238 When the majority of plants reached the V3/V4 growth stage, nicosulfuron was applied to one-
239 half of each row at a rate of 35 g ai ha^{-1} with a 0.25% (v/v) nonionic surfactant and 2.5% (v/v)
240 urea ammonium nitrate. This treatment was delivered using a pressurized CO_2 backpack sprayer
241 equipped with AI110025 nozzles (TeeJet Technologies, P.O. Box 7900, Wheaton, IL 60187)
242 spaced 51 cm apart on a 1.5-meter boom calibrated to apply 187 L ha^{-1} at 276 kPa. Popcorn

243 plants were evaluated ten days after nicosulfuron application. Injury was assessed visually on a
244 scale of 0-4, with 0 representing no injury and 4 representing plant death.

245 **2.8 Imputation and genome-wide association studies (GWAS)**

246 To evaluate the imputation accuracy of different methods, 1% of the GWAS Filtered set was
247 masked and imputed. Imputation was performed with FILLIN (Swarts et al. 2014) and Beagle
248 v5.1 (Browning et al. 2018). FILLIN was run using a haplotype block size of 4,096. Beagle was
249 run using an effective population size of 100,000 and an HP301 genetic map. The genetic map
250 for HP301 was constructed in *R/qtl* (Broman et al. 2003) using GBS data from the HP301 RILs
251 (Elshire et al. 2011), aligned to the HP301 v1 reference genome using the TASSEL 5 GBS v2
252 discovery pipeline. To compare the two imputation methods, the unmasked, masked, and
253 imputed marker sets were used as input for TASSEL's ImputationAccuracyPlugin, and error
254 rates were generated.

255 As the popcorn populations differ in kernel color and response to nicosulfuron, GWAS was
256 performed on both these phenotypes. Both GWAS analyses were performed using a weighted
257 MLM in TASSEL with the FILLIN-imputed marker set, a kinship matrix of normalized IBS
258 values, and a weight matrix of principal coordinates with high eigenvalues. A Bonferroni-
259 corrected p-value of 0.01 resulted in a -log(p) significance threshold of 7.3. Results of both
260 GWAS analyses were visualized using the *R* package *qqman* (Turner 2018).

261 **3 RESULTS**

262 **3.1 Germplasm**

263 To improve the genomic resources and investigate the genetic diversity of popcorn, GBS was
264 performed on 362 popcorn accessions. In GRIN, a total of 640 available accessions were listed
265 as popcorn. Accessions that were also labeled as other kernel types were removed, reducing the
266 count to 379. Then all accessions that appeared to be duplicates based on passport information
267 were removed, bringing the number of unique popcorn accessions down to 304. Of the
268 accessions with multiple kernel types listed, 37 were added back, as they were known popcorn
269 lines. Finally, an additional 21 popcorn landraces from CIMMYT were included, bringing the
270 total number of accessions to 362 (Table S1). Of these, 72 are listed as landraces, 109 as
271 cultivated material, and the remaining 181 as breeding material. In addition to the 362 popcorn
272 accessions, four previously sequenced dent inbreds were included to serve as outgroup lines:
273 B73, Mo17, PH207, and Oh43.

274 **3.2 Marker density and distribution**

275 From the GBS data, a Core Analysis set of 417,118 markers with an average minor allele
276 frequency of 0.169 was generated after passing SNP filtering and quality control. A Filtered
277 GWAS set of 200,101 markers with an average minor allele frequency of 0.216 was generated
278 by removing all SNPs with greater than 0.75 missing data in preparation for imputation. Of the
279 nucleotide substitutions from biallelic SNPs, adenine-guanine and thymine-cytosine transitions
280 were the most common (29.5% each), while adenine-thymine transversions were the least
281 common (7.7%). Of the Core Analysis set of markers, 327,876 (78.6%) were identified as
282 intragenic using the HP301 v1 gene models (Hufford et al. 2021), consistent with the observation
283 that SNP density increased from the centromeric to the telomeric regions (Supplementary Fig.

284 S1). Across the 10 maize chromosomes, the Core Analysis set of markers had an average marker
285 density of 197.3 SNPs per Mb, with the highest and lowest marker densities observed on
286 chromosomes 5 (216 SNPs per Mb) and 6 (184 SNPs per Mb), respectively (Table 1). The
287 Filtered GWAS set had an average marker density of 94.6 SNPs per MB, with the highest and
288 lowest densities on chromosomes 1 (108 SNPs per Mb) and 4 (74.7 SNPs per Mb), respectively.
289

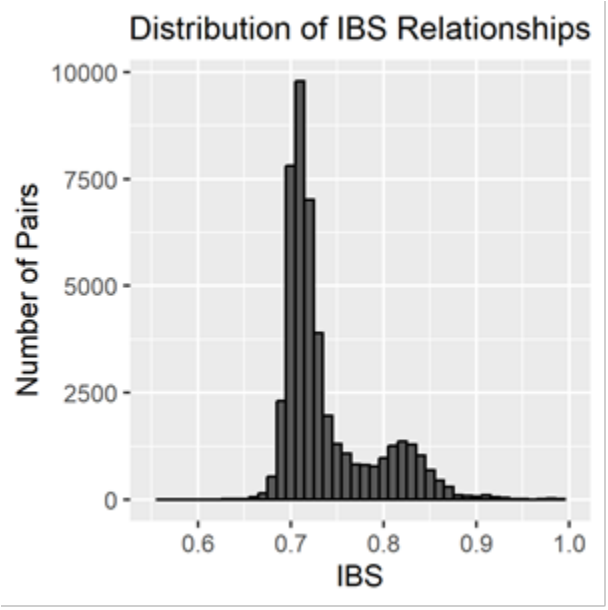
		Core Analysis Set			Filtered GWAS Set		
Chromosome	Chromosome Length (b)	Number of SNPs	SNPs per Mb	MAF	Number of SNPs	SNPs per Mb	MAF
1	305,741,490	64450	210.8	0.16807	32903	107.6	0.21527
2	245,520,863	51470	209.6	0.17452	24514	99.8	0.22231
3	240,329,036	47791	198.9	0.17328	24554	102.2	0.22337
4	251,015,817	41118	163.8	0.16409	18748	74.7	0.20173
5	221,904,911	47873	215.7	0.1712	23121	104.2	0.22609
6	177,672,499	32691	184.0	0.16488	14358	80.8	0.21071
7	180,265,189	35984	199.6	0.16534	15815	87.7	0.21834
8	177,329,642	34910	196.9	0.16316	16579	93.5	0.2199
9	164,337,916	31699	192.9	0.1707	16053	97.7	0.21045
10	150,435,773	29232	194.3	0.16794	13456	89.4	0.21866
Total	2,114,553,136	417218	197.3	0.16872	200101	94.6	0.21576

290 Table 1 SNP characteristics summary for the Core Analysis and Filtered GWAS SNP sets
291

292 **3.3 Population structure**

293 To identify relationships among accessions included in the panel, the distribution of IBS pairwise
294 comparisons for the 362 accessions was calculated using the Core Analysis set (Fig. 1). A
295 bimodal distribution was identified, with a large main peak centered on 0.72 and an additional
296 smaller peak centered on 0.82. These results indicate that although most accessions have a weak
297 relationship with other material in this panel, a subset appears to be more closely related.
298 The North American popcorn populations are comprised of accessions with different breeding
299 histories, geographic distributions, and selection pressures. An ADMIXTURE analysis was

300 performed on all taxa to formally test for population structure using one to five *K* populations.



301

302 Figure 1 Identity by state (IBS) distribution across 362 popcorn accessions

303

304 Cross-validation error was minimized at *K*=2 (Supplementary Fig. S2), and taxa were clustered
305 into two groups of similar size, with 147 individuals assigned to the first group and 215 assigned
306 to the second (Fig. 2, Table S1). Most taxa assigned to the first group are North American
307 Yellow Pearl Popcorns from breeding programs in the United States with known pedigrees and
308 generally fall into one of the previously described heterotic pools. However, several notable taxa
309 from Chile were assigned to this group, including CHZM 05 006, CHZM 06 004, CHZM 06 012,
310 and CHZM 07 097, all of which are members of the Curagua race. The remaining 23 members of
311 Curagua were assigned to the other group of popcorn, which will be referred to as the Pointed
312 and Latin American Popcorns. This group contains many diverse accessions, including all North
313 American Pointed Rice Popcorns (n=21), North American Early Popcorns (n=32), all Latin
314 American pointed and pearl popcorns accessions outside the four members of Curagua (n=98),
315 and nearly all lines developed outside of the Americas (n=64).

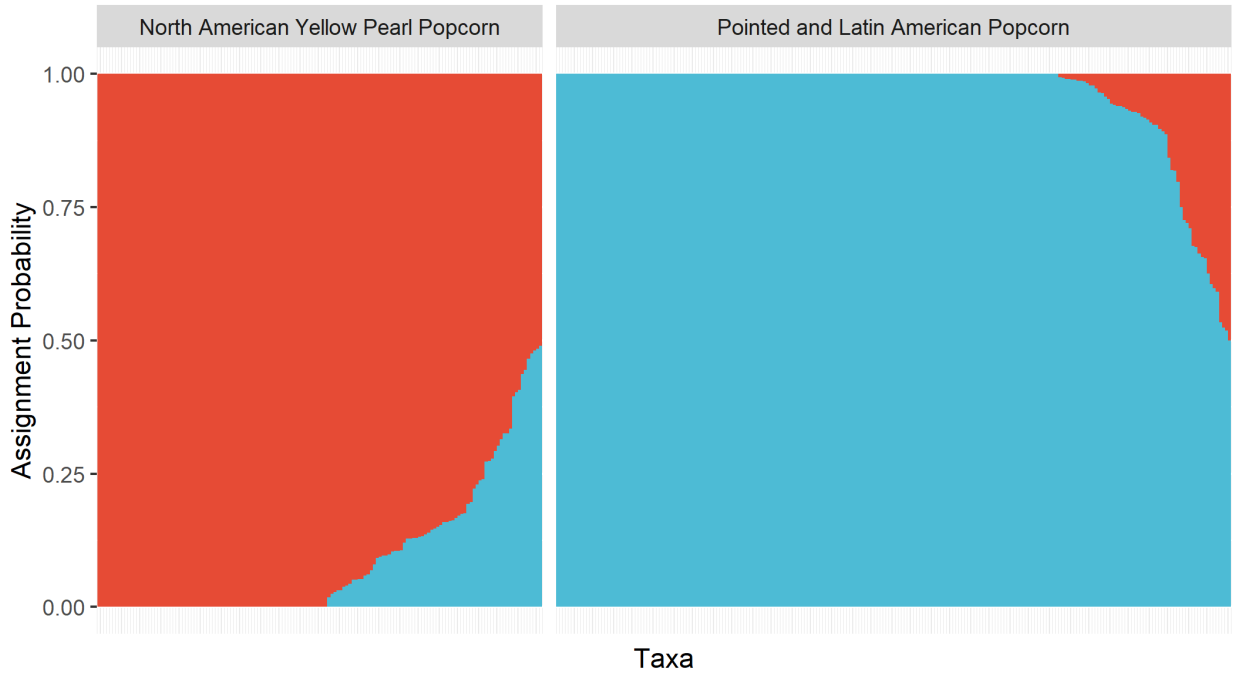


Figure 2 Bar plot of assignment values from ADMIXTURE analysis. Each bar corresponds to one of 362 popcorn accessions and is ordered by the likelihood of assignment. The complete list of assignments can be found in Table S1.

Since more than two popcorn groups have been reported previously in the literature, a second analysis was performed to ensure that the identification of additional popcorn groups was not limited by an insufficient number of related accessions. A combined dataset was generated after merging the 362 popcorn accessions with 2,284 accessions from the Ames Diversity Panel that were not categorized as dent (stiff stalk or non-stiff stalk). After minimizing the ADMIXTURE cross-validation error at K=15, the combined 2,646 accessions were assigned to 15 groups, with no additional popcorn groups identified. Although dent lines were excluded, 885 unclassified accessions were assigned to known public subgroups, including B73, B14, B52, Hy, Mo17, W22, Oh43, and WF9, demonstrating that dent material was still well-represented in the analysis. Notably, the analysis assigned the 17 teosinte inbred lines included in the Ames

Diversity Panel to the Pointed and Latin American Popcorn group. Thus, the teosinte inbred lines are most genetically similar to the Pointed and Latin American Popcorns, followed by North American Yellow Pearl Popcorns, then sweet corn, and finally dent and flint tropical and temperate material (Supplementary Fig. S3). Other notable taxa assigned to the Pointed and Latin American Popcorn group include accessions of Palomero Toluqueño, Reventador, Chapalote, Pororo, Avati Pichinga, and Pisankalla.

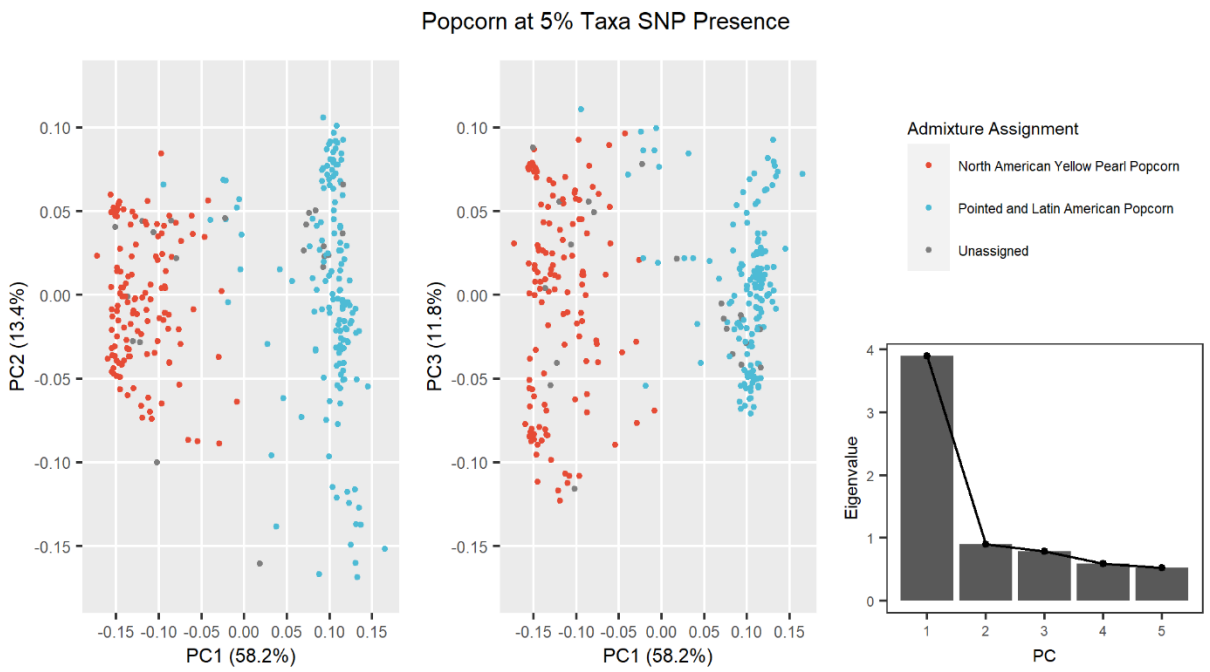


Figure 3 Principal coordinate analysis (PCoA) of 362 popcorn accessions estimated with the Core Analysis set and associated scree plot.

Principal coordinate analysis (PCoA) was also performed to investigate population structure and identify individual taxa grouping, and three of the coordinates explained 83.3% of the variation (Fig. 3). The first coordinate separates North American Yellow Pearl Popcorns from the Pointed and Latin American Popcorns, supporting the *K*=2 optimum number of clusters of the popcorn

ADMIXTURE analysis. The second coordinate separates the Pointed and Latin American Popcorns into three general divisions. Most of the North American Pointed Rice Popcorns cluster together near the bottom, with several accessions of Pisankalla. Next, most of the Latin Pointed Rice Popcorns group in the center with members of Curagua and Reventador. Finally, near the top, lowland tropical popcorn landraces such as Avati Pichinga, Pipoca, and Pororo are located together. The third coordinate separates the North American Yellow Pearls, with accessions from the South American heterotic group clustered near the bottom, those from Supergold clustered at the top, and lines developed by Iowa State's Popcorn Breeding Program located in the center. The neighbor-joining tree based on the distance matrix supported the population structure findings of the ADMIXTURE analysis and PCoA (Fig. 4). The major division of clades within the tree corresponded to the separation between North American Yellow Pearl Popcorns and Pointed and Latin American Popcorns. Several Chilean Curagua accessions fall within and basal to the North American Yellow Pearl clade, while the remainder are located within the Pointed and Latin American Popcorns. Two additional Latin American Taxa assigned to the North American Yellow Pearl Popcorn population, BRAZ 2832 and SONO 147, occur within the Supergold clade, indicating that they likely represent a recent movement of North American Yellow Pearl Popcorn germplasm (Fig. 4).

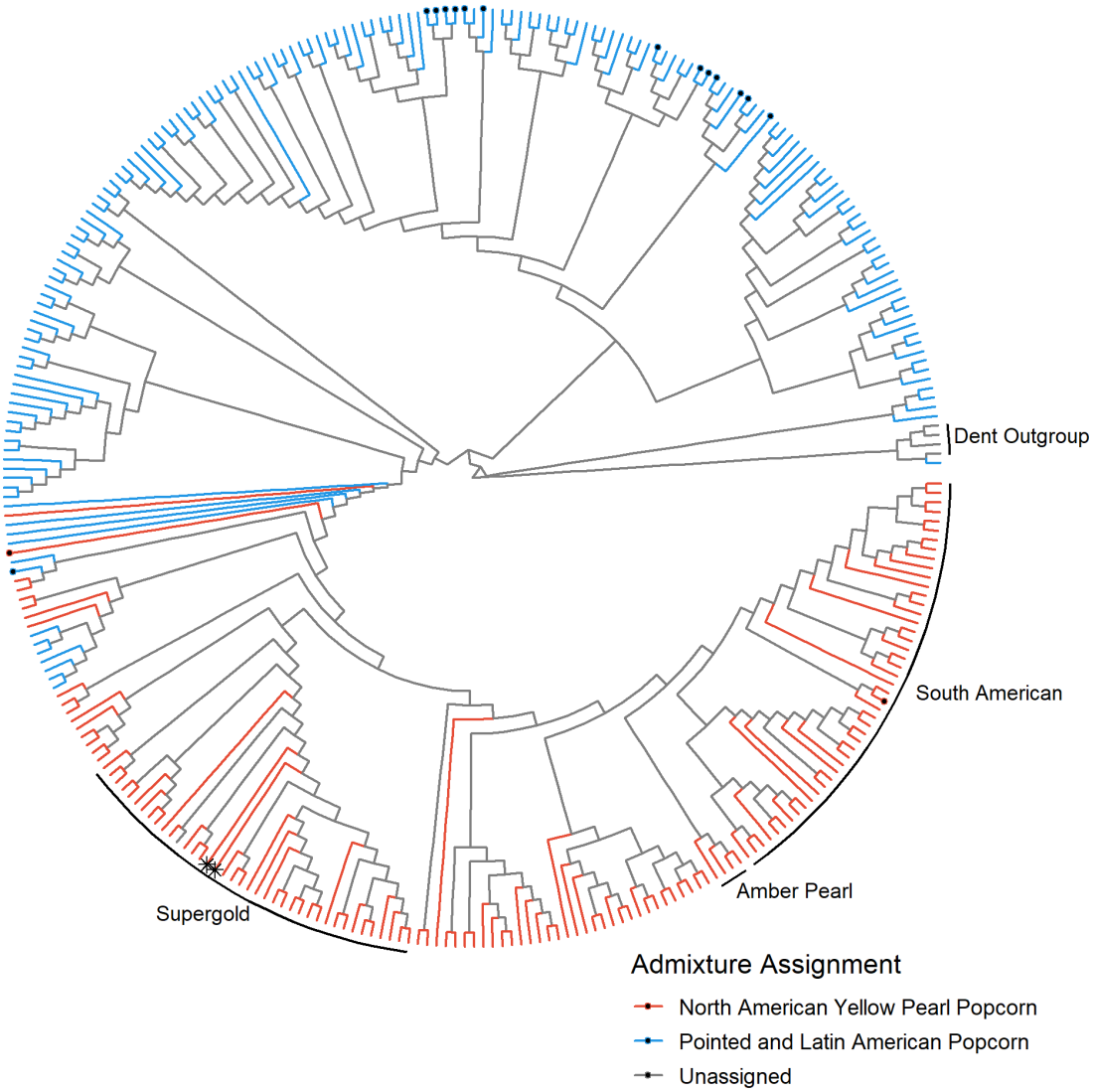


Figure 4 Neighbor-joining tree of 332 popcorn accessions and 4 dent accessions based on a modified Euclidean distance matrix calculated from the Core Analysis set. The three heterotic pools of North American Yellow Pearl Popcorns are labeled, along with the dent outgroup. Accessions SONO 147 and BRAZ 2832 are marked with an asterisk (∗). Accessions belonging to Curagua are marked with a dot (•).

3.4 Population genetics and allele diversity

To evaluate differences in allele frequencies and genetic diversity within popcorn germplasm, each population was evaluated for the proportion of monomorphic SNPs, minor allele frequencies (MAF), and LD decay. After filtering markers in each group to account for structural variation, monomorphism was evaluated. The proportion of monomorphic SNPs was considerably different between the two groups, with 5.5 times more monomorphic SNPs in the North American Yellow Pearl Popcorns (0.328) than in the Pointed and Latin American Popcorns (0.059). Additionally, there were differences in MAF between the two populations (Fig. 5).

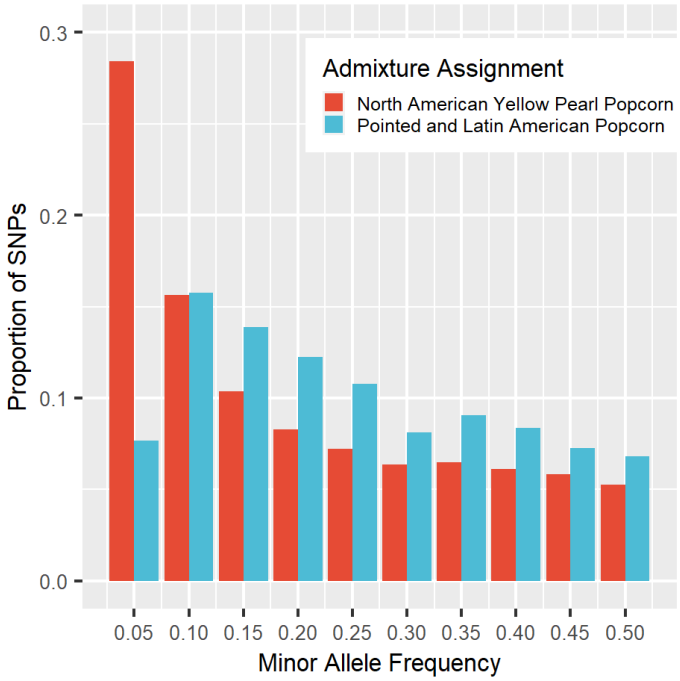


Figure 5 Minor allele frequency (MAF) distributions of the two popcorn populations. Y-axis shows the proportion of SNPs, as differing counts of monomorphic SNPs were removed from each population prior to calculating frequencies.

The relative frequency of SNPs with a MAF of 0.05 or less in North American Yellow Pearl Popcorns and Pointed and Latin American Popcorns were 28.4% and 7.7%, respectively. For all MAF greater than 0.05, the Pointed and Latin American Popcorns contained more SNPs than the North American Yellow Pearl Popcorns. Overall, the North American Yellow Pearl Popcorns contained more SNPs with low MAF than the Pointed and Latin American Popcorns.

Linkage disequilibrium decay also differed between the two populations (Fig. 6). Decay was low in the North American Yellow Pearl Popcorns, reaching a median r^2 of 0.3 within 10 – 99 Kb. In contrast, LD decayed rapidly in the Pointed and Latin American Popcorns, reaching a median r^2 of 0.1 within 10 – 99 bp. When compared with previous evaluations of LD decay in temperate and tropical populations (Romay et al. 2013, White et al. 2020), North American Yellow Pearl Popcorns exhibit slower LD decay than even the B73 heterotic subgroup (r^2 of ~0.25 within 10 – 99 Kb), while LD decay in the Pointed and Latin American Popcorns was slightly faster than in CIMMYT tropical germplasm (r^2 of 0.1 within 100 – 999 bp).

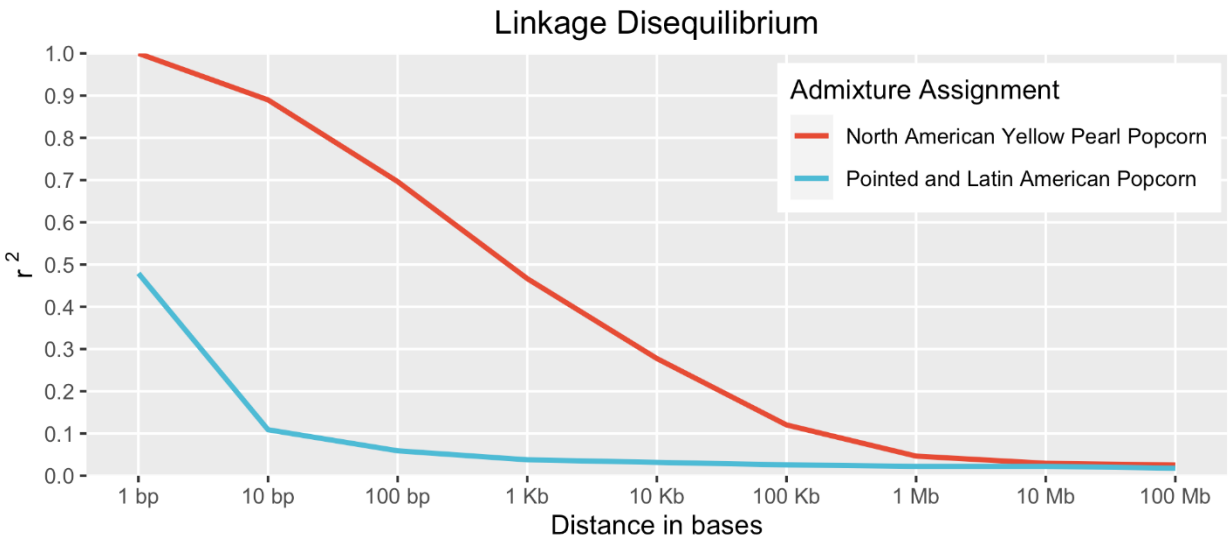


Figure 6 Median linkage disequilibrium (LD) decay of the two popcorn populations. Pairwise comparisons were binned by distance using logarithmically increasing bins.

399 **3.5 Genome-wide association studies**

400 A significant limitation of GWAS is the *p*-value correction required to account for multiple tests
401 performed (Tam et al. 2019). Reducing the number of markers tested is one way to ease the
402 stringency of the commonly used Bonferroni correction method. By removing all markers with
403 greater than 0.75 missing data, the resulting Filtered GWAS set reduced the number of markers
404 to approximately half of the Core Analysis set. Furthermore, since the imputation strategies
405 tested here rely on generating haplotypes from existing marker data, any reduction in missing
406 sites improves haplotype generation and subsequent imputation. Two commonly used imputation
407 strategies in maize are Beagle and FILLIN. Both were used to impute the Filtered GWAS set,
408 and error metrics were calculated for each. FILLIN and Beagle had overall error rates of 0.02321
409 and 0.08173, respectively. Because of the difference in error rates, only the FILLIN-imputed
410 marker set was used to perform GWAS.

411 The North American Yellow Pearl Popcorns and Pointed and Latin American Popcorns represent
412 two distinct populations with significant differences in population structure, genetic diversity,
413 and breeding history. Additionally, phenotypic differences are known between these two
414 populations, such as kernel color. North American Yellow Pearl Popcorns are typically yellow,
415 with only four white accessions in the panel (3.3%), while the Pointed and Latin American
416 Popcorns have a much higher frequency of white kernels or endosperm, with 111 accessions in
417 the panel (54.7%). Since kernel color is a simple trait that differs among the germplasm assessed
418 here, it was selected as a proof of concept to assess the utility of using this panel for GWAS (Fig.
419 7). Several significant SNPs were identified, some of which have been associated with kernel
420 color previously (Table S2). The SNP with the strongest association with kernel color is located
421 in *Y1*, which is known to affect the concentration of carotenoid pigments in the endosperm. The

SNP with the next strongest association with kernel color is located 200 bases away from Zm00027ab258670, a gene model predicted to have carotene desaturase activity. Three other peak SNPs were identified, none of which have been previously associated with kernel color. These results indicate that despite the known differences in population structure and phenotype frequencies, the panel can identify associations between loci and traits.

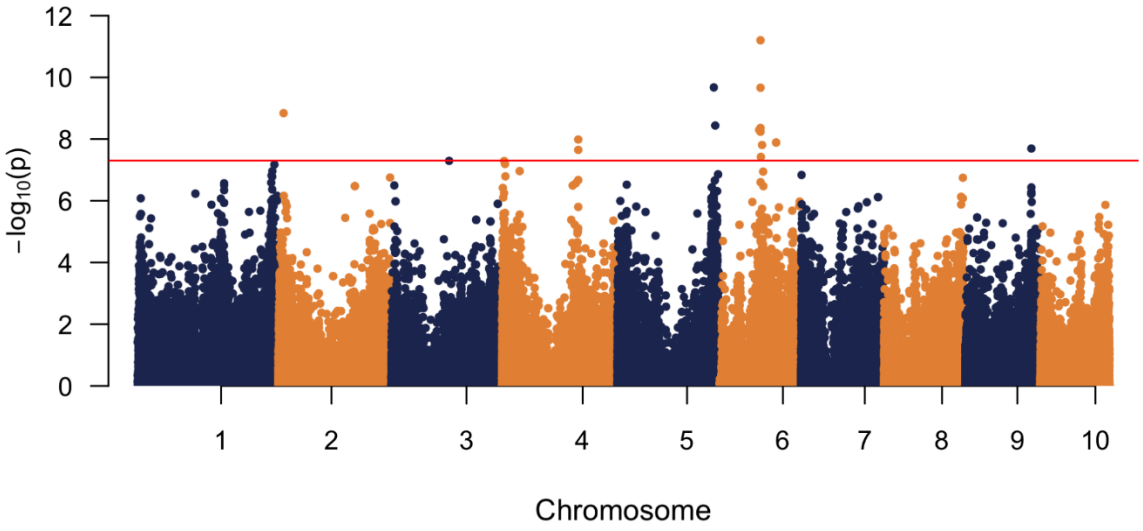


Figure 7 Manhattan plot of genome-wide association study (GWAS) of kernel color from the Filtered GWAS set. The red line represents the threshold after correcting for multiple testing using a Bonferroni correction.

A trait anecdotally associated with kernel color in popcorn is herbicide injury. Herbicide labels often exclude white popcorn from approved use, even when yellow popcorn is included (Loux et al. 2017). While the white color itself should have no bearing on injury, herbicide sensitivity differences between the two popcorn populations may have been reduced to kernel color for simplicity. In the herbicide trial, 4 of the 111 North American Yellow Pearl Popcorns (3.6%) and

437 32 of 178 Pointed and Latin American Popcorns (18%) showed typical nicosulfuron injury
438 (Table S3). Of the 32 injured Pointed and Latin American Popcorns, several are important
439 historical lines grown in the American Midwest, including Japanese Hulless, Australian Hulless,
440 H5505, IA DS 38-W, and IA DS 43-W. Notably, these pointed lines are all white and represent
441 much of the variation of the pointed rice popcorns traditionally grown in North America.
442 The Filtered GWAS set was used to map nicosulfuron injury. A total of nine SNPs representing
443 seven peaks were found to be significant (Fig. 8, Table S4). The SNP with the strongest
444 association with injury is located in *cl11433_2b*, a transcription factor associated with the
445 thylakoid. The next strongest association was with a subunit of NADH dehydrogenase. Other
446 SNPs with statistically significant associations include a predicted protein kinase, the vacuole-
447 associated phospholipase *pco139881*, a locus 1.3 kb upstream from a predicted aminoacyl-tRNA
448 synthetase, the cytochrome p450 *CYP72A27*, and a predicted monooxygenase
449 glycosyltransferase. Although these genes have not previously been implicated in nicosulfuron
450 injury, many of the classes to which they belong are associated with herbicide tolerance.

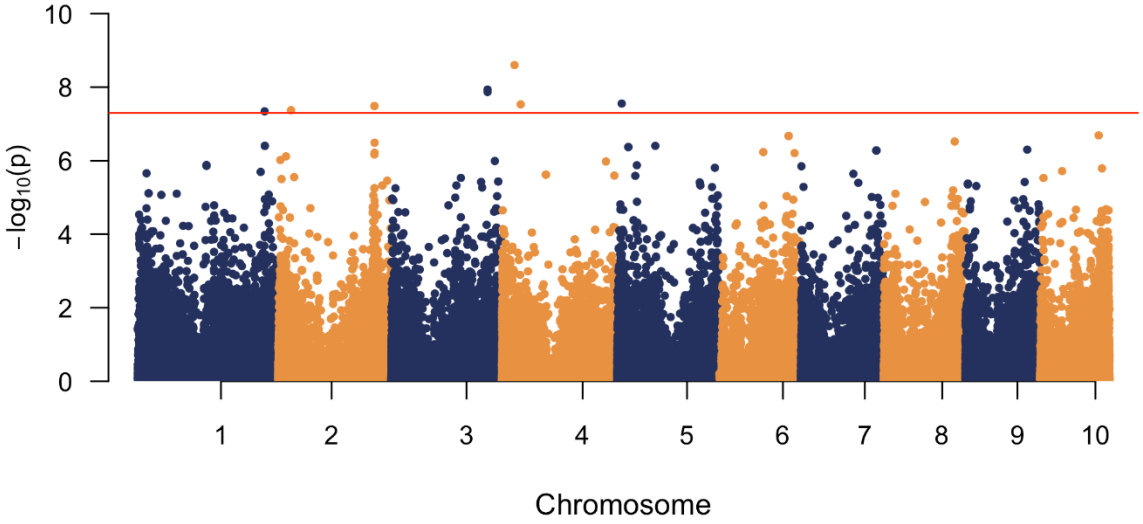


Figure 8 Manhattan plot of genome-wide association study (GWAS) of nicosulfuron injury from the Filtered GWAS set. The red line represents the threshold after correcting for multiple testing using a Bonferroni correction.

4 DISCUSSION

4.1 Population Structure

The results of IBS, ADMIXTURE, PCoA, and the neighbor-joining tree support two populations of popcorn in the panel investigated here: the North American Yellow Pearl Popcorns and the Pointed and Latin American Popcorns. Both the PCoA and neighbor-joining tree indicate a degree of sub-grouping within each group, but these subgroups were not significantly different based on the genomic data. Within the North American Yellow Pearl Popcorns, differences in population structure of the Supergold and South American heterotic groups were apparent, and there was a minor grouping effect on lines originating from Iowa State's Popcorn Breeding Program. Divisions within the Pointed and Latin American Popcorns resulted in three general subgroups – North American Pointed Rice Popcorns, Latin Pointed Rice and highland popcorn landraces, and lowland tropical popcorn landraces. It is important to note that not all individuals typically assigned to one of these subgroups clustered with their subgroup, though the majority do. Although sub-groups were identified in both populations, they are not comparable in magnitude to the division between North American Yellow Pearl Popcorns and Pointed and Latin American Popcorns. Therefore, two groups are proposed here that explain most of the variation in population structure in this popcorn diversity panel.

Foundational work on the phylogenetic relationships of North American popcorn identified more than two groups of popcorn. After evaluating 56 popcorn populations for morphological traits, isozymes, and SSRs, six major divisions were previously identified (Santacruz-Varela et al. 2004). Two groups of pointed rice popcorn of North American and Latin American origin were proposed, followed by the related North American Early Popcorns. A single population of North American Yellow Pearl Popcorns was identified, with minimal divisions between the heterotic

478 groups. Finally, two additional groups of Latin America were identified, one corresponding to
479 lowland races of Argentina, Brazil, and Paraguay, and the other composed of various accessions
480 from Chile, Uruguay, and Mexico.
481 Besides the number of lines, a major difference between previous studies and the results
482 presented here is that morphological traits were not included when determining phylogenetic
483 relationships and population structure. All traits are composed of genotypic and environmental
484 effects, and many of the vegetative and phenological traits included in previous analyses of
485 popcorn are known to be affected by photoperiod sensitivity, including plant height, ear height,
486 leaf number, and days to flowering (Fei et al. 2022). As few as four genomic regions have been
487 identified that control photoperiod responses in these traits (Coles et al. 2010). Therefore,
488 including these traits in measures of phylogenetic divergence likely does not reflect genomic
489 differences between populations and could be heavily influenced by photoperiod sensitivity. This
490 is supported by the conclusion that the six previously identified popcorn populations were
491 primarily assigned based on results of the morphological characterization (Santacruz-Varela et
492 al. 2004). The inclusion of morphological traits that do not reflect genome-wide differences
493 explains the discrepancy between the six populations of popcorn identified previously and the
494 two populations presented here.
495 Investigation into the genetic diversity and population structure of CIMMYT accessions and
496 Chinese germplasm has identified distinct popcorn populations in their panels. A panel
497 composed of teosinte, popcorn landraces, non-popcorn landraces, North American Yellow Pearl
498 Popcorns, and elite CIMMYT inbreds was used to identify loci associated with temperate and
499 tropical adaptation (Li et al. 2019). This panel identified two groups of popcorn, one
500 corresponding to the North American Yellow Pearl Popcorns and the other associated with

501 landraces from Latin America. The genetic diversity and population structure within a popcorn
502 collection of Chinese accessions, CIMMYT landraces, and inbreds from the United States were
503 recently characterized (Yu et al. 2021). In this study, popcorn accessions were assigned to one of
504 three groups – Latin American landraces, North American Yellow Pearl Popcorns, and recently
505 developed inbreds from breeding programs, likely of North American Yellow Pearl origin. Based
506 on these studies and the findings presented here, North American Yellow Pearl Popcorns have
507 consistently been shown to constitute a genetically distinct pool of germplasm compared to Latin
508 American landraces.
509 In contrast with the uniform separation of North American Yellow Pearl Popcorns from other
510 types of maize, the assignment of the Pointed and Latin American Popcorns is not as consistent.
511 Single-group assignment of popcorn landraces of Latin America has been previously reported
512 when compared with Chinese germplasm as discussed above (Yu et al. 2021), Brazilian popcorn
513 inbreds (Vittorazzi et al. 2018), and highland and lowland Argentine landraces (Bracco et al.
514 2012). However, an investigation of Latin American and Caribbean landraces identified sixteen
515 populations, three of which included popcorn (Bedoya et al. 2017). The first of the three
516 populations contained Palomero Toluqueño, Chapalote, and Reventador, all of which are
517 considered primitive popcorns of Northern Mexico. The second group was related to the first and
518 contained Canguil, Confite Puneño, and Pisankalla, all of which are pointed. The third group was
519 unrelated to the first two and contained lowland Avati popcorn. More recently, an analysis of
520 575 popcorn landraces from Latin America identified nine genetic clusters using discriminant
521 analysis of principal components (DAPC) without considering a priori groups (De Almeida Silva
522 et al. 2020). However, it is important to consider differences in methods when comparing these
523 findings.

524 Most popcorn groups have been identified using model-based clustering approaches such as
525 STRUCTURE (Pritchard et al. 2000) or ADMIXTURE. Furthermore, model-based clustering
526 methods assign individuals to populations relative to the entire panel – populations identified by
527 an initial clustering analysis can be further divided into sub-groups by subsequent clustering
528 analyses, as with the American and Caribbean landraces discussed above. Additionally, methods
529 based on PCA, like DAPC, tend to provide higher estimates of the total number of populations
530 than model-based clustering approaches (Intarapanich et al. 2009, Linck & Battey 2019) and are
531 especially sensitive to the influence of a priori vs. de novo groupings (Miller et al. 2020).
532 Because of these considerations, a single assignment by the model-based clustering method
533 ADMIXTURE on the Core Analysis set was used to assess population structure for the entire
534 panel. The findings reported here largely agree with similar approaches to estimate population
535 structure in popcorn, and differences with other reports largely stem from assignment methods.

536 **4.2 Genetic diversity**

537 The Pointed and Latin American Popcorns contain a variety of accessions, including pointed
538 popcorns from the American Midwest. It is unlikely that the genetic similarities between lines of
539 pointed popcorn from North America and Latin America indicate a recent introduction or
540 transfer of germplasm between continents, as the lines constituting this group were
541 geographically widely distributed. Instead, these similarities may indicate a pre-Columbian
542 interchange of popcorn between North and South America, as has been previously suggested
543 (Bedoya et al. 2017). Additionally, the genetic similarity may reflect reduced admixture with
544 other landraces due to the high frequency of the dent-sterile *Ga1-S* allele in North and South
545 American popcorn (Goodman et al. 2021). This aligns with the finding that popcorn landraces
546 tend to group separately from other maize types, as discussed above.

547 North American Yellow Pearl Popcorns share genetic similarities with several Curagua
548 accessions from Chile. Genetic similarity between United States varieties and South American
549 material has been noted before (Vigouroux et al. 2008, van Heerwaarden et al. 2010). However,
550 this has generally been attributed to the post-Columbian movement of germplasm from the
551 United States to South America. Many Chilean, Argentine, and Brazilian races are of United
552 States origin, including Dulce Golden Bantam, Hickory King, and Dente Branco. The presence
553 of Curagua accessions in the North American Yellow Pearls is evidence that intercontinental
554 movement from South to North also occurred.
555 The North American Yellow Pearl Popcorns have a much higher LD and proportion of
556 monomorphic markers than Pointed and Latin American Popcorns and other populations of
557 maize (Romay 2013). North American Yellow Pearl Popcorns also exhibit higher IBS values
558 when compared with other North American Yellow Pearl Popcorns and lower minor allele
559 frequencies, indicating a high rate of inbreeding and lower genetic variation. Considering the few
560 Curagua accessions clustered among the North American Yellow Pearls (Fig. 4), this reduced
561 genome variation is consistent with a small founder population and fewer meiotic events relative
562 to the considerably older Pointed and Latin American Popcorns. The genetic variation within the
563 three accessions of Curagua assigned to the North American Yellow Pearls represents only a
564 portion of the total variation present in all members of the Curagua race. If members of Curagua
565 were transported to New England by sailors, as previously suggested (Smith 1999), they would
566 have likely contained a limited gene pool, and subsequent inbreeding beginning in the 1940s
567 would have exacerbated the limited genetic diversity. However, it appears that after development
568 in the United States, some of this germplasm returned to Latin America, as evidenced by the
569 SONO 147 and BRAZ 2832 accessions (Fig. 4). Although little additional information is known

570 about BRAZ 2832, the passport data for SONO 147 (National Genetic Resources Program,
571 2022) contains the alternate name "Reventador Gringo", suggesting that growers associated this
572 population with non-native material.

573 **4.3 Kernel color, popcorn populations, and nicosulfuron injury**

574 The product of the *Y1* gene catalyzes the first step in the maize carotenoid biosynthesis pathway.
575 A SNP in this gene has been identified as a causal mutation that results in a Thr to Asn
576 substitution (Fu et al. 2013). The peak SNP identified in the GWAS is tightly linked with the
577 causal SNP, separated by 346 bases. Another SNP occurs close to a gene model predicted to
578 have carotene desaturase activity and is located 3 Mb away from a locus previously associated
579 with popcorn flake color in CIMMYT germplasm (Li et al. 2021). Given the structural variation
580 between popcorn germplasm and most material used to evaluate kernel color, it is plausible that
581 additional loci may be identified outside the core genome (Lu et al. 2015, Hufford et al. 2021).
582 The three remaining SNPs reported here have not been previously associated with kernel color,
583 although two occur in known genes, *wrky11* and *nc3*, and the other in an uncharacterized gene.
584 Further investigation is necessary to determine whether these additional associations represent
585 biologically meaningful relationships or spurious correlations from population structure.
586 Recent studies have been unable to identify differences in herbicide sensitivity among several
587 (i.e., up to eight entries) commercial white and yellow popcorn hybrids (Barnes et al. 2020a;
588 Barnes et al. 2020b). However, given the unknown origin of the hybrid parents used in these
589 studies and the prevalence of North American Yellow Pearl Popcorns in commercial germplasm,
590 these hybrids were likely selected using kernel color alone and do not represent the two North
591 American popcorn populations. The results presented here show significant differences in the
592 two popcorn populations for susceptibility to nicosulfuron. Kernel color has no bearing on

593 herbicide injury and demonstrates the importance of population structure in genetic analyses.
594 Kernel color represents a major phenotypic difference between the generally nicosulfuron-
595 tolerant North American Yellow Pearl Popcorns and the more susceptible Pointed and Latin
596 American Popcorns.
597 Nicosulfuron, along with other sulfonylurea herbicides, inhibits acetolactate synthase (ALS), the
598 first enzyme in the biosynthesis of branched-chain amino acids (BCAAs). Although encoded by
599 the nuclear genome, ALS is localized in the chloroplast (Mazur et al. 1987). Along with other
600 enzymes involved in BCAA biosynthesis, ALS interacts with many substrates and products of
601 cellular respiration, including pyruvate, NAD, and FAD (Duggleby et al. 2008). While target-site
602 resistance of sulfonylurea herbicides has been demonstrated in maize (Anderson & Georgeson
603 1989; Li et al. 2020), non-target site resistance is more common and complex (Délye 2013).
604 Non-target site resistance involves initial detoxification, often in the form of cytochrome p450s
605 and other oxidases, followed by conjugation of thiols or glucosyl groups, and subsequent
606 transport and degradation in the vacuole or extracellular spaces (Yuan et al. 2007).
607 In sweet and dent corn, detoxification of several post-emergent herbicides, including
608 nicosulfuron, is driven largely by *CYP81A9*, a cytochrome p450 produced by the *Nsf1* locus
609 (Nordby et al. 2008, Choe and Williams 2020). Although this does not appear to be the case with
610 nicosulfuron response in popcorn, other candidate genes were identified. Since the pool of
611 available NADH in oxidative phosphorylation is driven largely by pyruvate, variation in NADH
612 dehydrogenase may allow plants to tolerate changes in pyruvate as ALS becomes bound by
613 nicosulfuron. Additionally, variation in glycosylation and changes in the vacuole is consistent
614 with non-target site resistance. Furthermore, altered amino acid synthesis and phosphorylation
615 may be involved in the alternate metabolic flux of some BCAAs (Joshi et al. 2010). Finally, as

616 many of these processes occur within, or are related to, the chloroplast, changes in the
617 transcription factors of chloroplast-associated genes would be expected.
618 Although *CYP72A27* is a cytochrome p450, its identification here is somewhat unexpected, as it
619 belongs to the CYP clan 72 group of cytochrome p450s. Members of this clan are generally
620 associated with secondary metabolism, while clan 71 enzymes, such as *CYP81A9*, are
621 responsible for herbicide detoxification (Prall et al. 2016; Brazier-Hicks et al. 2022). However,
622 sulfonylurea herbicide metabolism via clan 72 enzymes has been shown in other crops, such as
623 *CYP72A31* in *Oryza indica* (Saika et al. 2014) and *CYP749A16* in *Gossypium hirsutum* (Thyssen
624 et al. 2018). Furthermore, several members of CYP72 were shown to be differentially expressed
625 in nicosulfuron tolerant and susceptible lines of maize when treated with the herbicide (Liu et al.
626 2015). Alternatively, the role of *CYP72A27* in detoxifying nicosulfuron may be unique to the
627 Pointed and Latin American Popcorns and simply not previously reported. However, it is
628 possible that rather than directly detoxifying nicosulfuron, *CYP72A27* and other genes identified
629 here respond more like safener-induced enzymes, conferring protection via a stress signaling
630 pathway (Riechers et al. 2010). Finally, as with other candidate genes identified using GWAS,
631 *CYP72A27* may have a spurious correlation with nicosulfuron response. Determining the roles
632 and mechanisms of the genes presented here with nicosulfuron tolerance would require further
633 investigation, including the phenotyping and genotyping of additional members of the Pointed
634 and Latin American Popcorns.

5 CONCLUSION

High-quality genetic resources are important tools for maize breeding programs. A collection of 362 popcorn accessions were analyzed here using 417,218 SNPs. Two distinct populations of popcorn were identified that exhibit genetic and phenotypic differences. North American Yellow Pearl Popcorns constitute a pool of germplasm with reduced genetic diversity and greater rates of inbreeding when compared with the Pointed and Latin American Popcorns. Kernel color frequencies were shown to differ between the two populations, along with sensitivity to nicosulfuron. Novel candidate genes responsible for nicosulfuron tolerance were identified in popcorn. The genomic characterization provided here should be incorporated into popcorn breeding programs to direct improvement and accelerate the rate of genetic gain. Additionally, this collection of popcorn germplasm and genetic data can be used by the maize research community to explore the genetic architecture of traits in popcorn and be compared with loci identified in other populations of maize.

648 **SUPPLEMENTAL MATERIAL**

649 Supplemental Table S1 ADMIXTURE assignment probabilities of 362 popcorn accessions by
650 population.

651 Supplemental Table S2 Significant markers identified by GWAS of kernel color.

652 Supplemental Table S3 Nicosulfuron injury of 294 accessions, including 111 North American
653 Yellow Pearl Popcorns, 178 Pointed and Latin American Popcorns, and five controls.

654 Supplemental Table S4 Significant markers identified by GWAS of nicosulfuron injury.

655 Supplemental Figure S1 SNP density and distribution from the Core Analysis set across
656 chromosomes at a 1Mb window size.

657 Supplemental Figure S2 Admixture cross-validation plot for 362 popcorn accessions using the
658 Core Analysis set.

659 Supplemental Figure S3 Neighbor-joining tree of 150 maize and 17 teosinte accessions based on
660 a modified Euclidean distance matrix calculated from the Core Analysis set.

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AUTHOR CONTRIBUTIONS

Madsen Sullivan: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. Martin M. Williams II: Conceptualization, Methodology, Resources, Writing – review & editing. Anthony J. Studer: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome research*, *19*(9), 1655-1664.

Anderson, P. C., & Georgeson, M. (1989). Herbicide-tolerant mutants of corn. *Genome*, *31*(2), 994-999.Li, Y., Zhu, J., Wu, H., Liu, C., Huang, C., Lan, J., ... & Xie, C. (2020). Precise base editing of non-allelic acetolactate synthase genes confers sulfonylurea herbicide resistance in maize. *The Crop Journal*, *8*(3), 449-456.

Barnes, E. R., Lawrence, N. C., Knezevic, S. Z., Irmak, S., Rodriguez, O., & Jhala, A. J. (2020). Dose response of yellow and white popcorn hybrids to glyphosate, a premix of 2, 4-D choline and glyphosate, or dicamba. *Agronomy Journal*, *112*(4), 2956-2967.

Barnes, E. R., Lawrence, N. C., Knezevic, S. Z., Rodriguez, O., Irmak, S., & Jhala, A. J. (2020). Weed control and response of yellow and white popcorn hybrids to herbicides. *Agronomy Journal*, *112*(1), 458-469.

Bautista-Ramírez, E., Santacruz-Varela, A., Córdova-Téllez, L., Muñoz Orozco, A., López-Sánchez, H., & Esquivel-Esquivel, G. (2020). Yield and expansion capacity of the corn grain in the Palomero Toluqueño race. *Revista mexicana de ciencias agrícolas*, *11*(7), 1607-1618.

Bedoya, C. A., Dreisigacker, S., Hearne, S., Franco, J., Mir, C., Prasanna, B. M., ... & Warburton, M. L. (2017). Genetic diversity and population structure of native maize populations in Latin America and the Caribbean. *PloS one*, *12*(4), e0173488.

Bracco, M., Lia, V. V., Hernández, J. C., Poggio, L., & Gottlieb, A. M. (2012). Genetic diversity of maize landraces from lowland and highland agro-ecosystems of Southern South America: implications for the conservation of native resources. *Annals of Applied Biology*, *160*(3), 308-321.

Brazier-Hicks, M., Franco-Ortega, S., Watson, P., Rougemont, B., Cohn, J., Dale, R., ... & Edwards, R. (2022). Characterization of Cytochrome P450s with Key Roles in Determining Herbicide Selectivity in Maize. *ACS omega*.

Broman, K. W., Wu, H., Sen, Ś., & Churchill, G. A. (2003). R/qtl: QTL mapping in experimental crosses. *bioinformatics*, *19*(7), 889-890.

Browning, B. L. (2018). Beagle 5.0. *University of Washington, Division of Medical Genetics*.

Brunson, A. M. (1937). Popcorn breeding.

Buckler, E. S., Holland, J. B., Bradbury, P. J., Acharya, C. B., Brown, P. J., Browne, C., ... & McMullen, M. D. (2009). The genetic architecture of maize flowering time. *Science*, *325*(5941), 714-718.

Choe, E., & Williams, M. M. (2020). Expression and comparison of sweet corn CYP81A9s in relation to nicosulfuron sensitivity. *Pest Management Science*, *76*(9), 3012-3019.

Coles, N. D., McMullen, M. D., Balint-Kurti, P. J., Pratt, R. C., & Holland, J. B. (2010). Genetic control of photoperiod sensitivity in maize revealed by joint multiple population analysis. *Genetics*, *184*(3), 799-812.

Cook, J. P., McMullen, M. D., Holland, J. B., Tian, F., Bradbury, P., Ross-Ibarra, J., ... & Flint-Garcia, S. A. (2012). Genetic architecture of maize kernel composition in the nested association mapping and inbred association panels. *Plant physiology*, *158*(2), 824-834.

Da Fonseca, R. R., Smith, B. D., Wales, N., Cappellini, E., Skoglund, P., Fumagalli, M., ... & Gilbert, M. T. P. (2015). The origin and evolution of maize in the Southwestern United States. *Nature plants*, *1*(1), 1-5.

De Almeida Silva, N. C., Vidal, R., Bernardi Ogliari, J., E Costich, D., & Chen, J. (2020). Relationships among American popcorn and their links with landraces conserved in a microcenter of diversity. *Genetic Resources and Crop Evolution*, *67*(7), 1733-1753.

Délye, C. (2013). Unravelling the genetic bases of non-target-site-based resistance (NTSR) to herbicides: a major challenge for weed science in the forthcoming decade. *Pest management science*, *69*(2), 176-187.

Duggleby, R. G., McCourt, J. A., & Guddat, L. W. (2008). Structure and mechanism of inhibition of plant acetohydroxyacid synthase. *Plant Physiology and Biochemistry*, *46*(3), 309-324.

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PloS one*, *6*(5), e19379.

Erwin, A. T. (1950). The origin and history of pop corn. *Economic Botany*, *4*(3), 294-299.

Fei, J., Jiang, Q., Guo, M., Lu, J., Wang, P., Liu, S., ... & Guan, S. (2022). Fine Mapping and Functional Research of Key Genes for Photoperiod Sensitivity in Maize. *Frontiers in Plant Science*, *13*.

Fu, Z., Chai, Y., Zhou, Y., Yang, X., Warburton, M. L., Xu, S., ... & Yan, J. (2013). Natural variation in the sequence of PSY1 and frequency of favorable polymorphisms among tropical and temperate maize germplasm. *Theoretical and Applied Genetics*, *126*(4), 923-935.

Gage, J. L., Monier, B., Giri, A., & Buckler, E. S. (2020). Ten years of the maize nested association mapping population: impact, limitations, and future directions. *The Plant Cell*, *32*(7), 2083-2093.

Glaubitz, J. C., Casstevens, T. M., Lu, F., Harriman, J., Elshire, R. J., Sun, Q., & Buckler, E. S. (2014). TASSEL-GBS: a high capacity genotyping by sequencing analysis pipeline. *PloS one*, *9*(2), e90346.

Goodman, M. M., Jones, Z. G., Sanchez, G. J., & Kermicle, J. L. (2021). Maize Cross Incompatibility and the Promiscuous Ga1-m Allele. *Plant breeding reviews*, *44*, 31-56.

Hufford, M. B., Seetharam, A. S., Woodhouse, M. R., Chougule, K. M., Ou, S., Liu, J., ... & Dawe, R. K. (2021). De novo assembly, annotation, and comparative analysis of 26 diverse maize genomes. *Science*, *373*(6555), 655-662.

Intarapanich, A., Shaw, P. J., Assawamakin, A., Wangkumhang, P., Ngamphiw, C., Chaichoompu, K., ... & Tongsima, S. (2009). Iterative pruning PCA improves resolution of highly structured populations. *BMC bioinformatics*, *10*(1), 1-17.

Joshi, V., Joung, J. G., Fei, Z., & Jander, G. (2010). Interdependence of threonine, methionine and isoleucine metabolism in plants: accumulation and transcriptional regulation under abiotic stress. *Amino acids*, *39*(4), 933-947.

Kantety, R. V., Zeng, X., Bennetzen, J. L., & Zehr, B. E. (1995). Assessment of genetic diversity in dent and popcorn (Zea mays L.) inbred lines using inter-simple sequence repeat (ISSR) amplification. *Molecular breeding*, *1*(4), 365-373.

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature methods*, *9*(4), 357-359.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., ... & Durbin, R. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics*, *25*(16), 2078-2079.

Li, J., Chen, G. B., Rasheed, A., Li, D., Sonder, K., Zavala Espinosa, C., ... & Li, H. (2019). Identifying loci with breeding potential across temperate and tropical adaptation via EigenGWAS and EnvGWAS. *Molecular ecology*, *28*(15), 3544-3560.

Li, J., Li, D., Espinosa, C. Z., Pastor, V. T., Rasheed, A., Rojas, N. P., ... & Li, H. (2021). Genome-wide analyses reveal footprints of divergent selection and popping-related traits in CIMMYT's maize inbred lines. *Journal of experimental botany*, *72*(4), 1307-1320.

Linck, E., & Battey, C. J. (2019). Minor allele frequency thresholds strongly affect population structure inference with genomic data sets. *Molecular Ecology Resources*, *19*(3), 639-647.

Liu, X., Xu, X., Li, B., Wang, X., Wang, G., & Li, M. (2015). RNA-seq transcriptome analysis of maize inbred carrying nicosulfuron-tolerant and nicosulfuron-susceptible alleles. *International Journal of Molecular Sciences*, *16*(3), 5975-5989.

Loux, M. M., Doohan, D., Dobbels, A. F., Johnson, W. G., Young, B. G., Legleiter, T. R., & Hager, A. (2017). Weed control guide for Ohio, Indiana and Illinois.

Lu, F., Romay, M. C., Glaubitz, J. C., Bradbury, P. J., Elshire, R. J., Wang, T., ... & Buckler, E. S. (2015). High-resolution genetic mapping of maize pan-genome sequence anchors. *Nature communications*, *6*(1), 1-8.

Matz, S. A. (1984). Snacks based on popcorn. In *Snack food technology* (pp. 138-149). Springer, Dordrecht.

Matz, Samuel A. "Snacks based on popcorn." *Snack food technology*. Springer, Dordrecht, 1984. 138-149.

Mazur, B. J., Chui, C. F., & Smith, J. K. (1987). Isolation and characterization of plant genes coding for acetolactate synthase, the target enzyme for two classes of herbicides. *Plant Physiology*, *85*(4), 1110-1117.

Merrill, W. L., Hard, R. J., Mabry, J. B., Fritz, G. J., Adams, K. R., Roney, J. R., & MacWilliams, A. C. (2009). The diffusion of maize to the southwestern United States and its impact. *Proceedings of the National Academy of Sciences*, *106*(50), 21019-21026.

Miller, J. M., Cullingham, C. I., & Peery, R. M. (2020). The influence of a priori grouping on inference of genetic clusters: simulation study and literature review of the DAPC method. *Heredity*, *125*(5), 269-280.

Nordby, J. N., Williams, M. M., Pataky, J. K., Riechers, D. E., & Lutz, J. D. (2008). A common genetic basis in sweet corn inbred Cr1 for cross sensitivity to multiple cytochrome P450-metabolized herbicides. *Weed Science*, *56*(3), 376-382.

Okonechnikov, K., Conesa, A., & García-Alcalde, F. (2016). Qualimap 2: advanced multi-sample quality control for high-throughput sequencing data. *Bioinformatics*, *32*(2), 292-294.

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, *20*(2), 289-290.

Peiffer, J. A., Romay, M. C., Gore, M. A., Flint-Garcia, S. A., Zhang, Z., Millard, M. J., ... & Buckler, E. S. (2014). The genetic architecture of maize height. *Genetics*, *196*(4), 1337-1356.

Prall, W., Hendy, O., & Thornton, L. E. (2016). Utility of a phylogenetic perspective in structural analysis of CYP72A enzymes from flowering plants. *PloS one*, *11*(9), e0163024.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945-959.

Riechers, D. E., Kreuz, K., & Zhang, Q. (2010). Detoxification without intoxication: herbicide safeners activate plant defense gene expression. *Plant Physiology*, *153*(1), 3-13.

Romay, M. C., Millard, M. J., Glaubitz, J. C., Peiffer, J. A., Swarts, K. L., Casstevens, T. M., ... & Gardner, C. A. (2013). Comprehensive genotyping of the USA national maize inbred seed bank. *Genome biology*, *14*(6), 1-18.

Saika, H., Horita, J., Taguchi-Shiobara, F., Nonaka, S., Nishizawa-Yokoi, A., Iwakami, S., ... & Toki, S. (2014). A novel rice cytochrome P450 gene, CYP72A31, confers tolerance to

acetolactate synthase-inhibiting herbicides in rice and Arabidopsis. *Plant physiology*, *166*(3), 1232-1240.

Santacruz-Varela, A., Widrlechner, M. P., Ziegler, K. E., Salvador, R. J., Millard, M. J., & Bretting, P. K. (2004). Phylogenetic relationships among North American popcorns and their evolutionary links to Mexican and South American popcorns. *Crop Science*, *44*(4), 1456-1467.

Serna-Saldivar, S. O. (2022). Popcorn and Other Puffed Grains. In *Snack Foods* (pp. 201-220). CRC Press.

Smith, A. F. (1999). *Popped culture: A social history of popcorn in America*. Univ of South Carolina Press.

Swarts, K., Li, H., Romero Navarro, J. A., An, D., Romay, M. C., Hearne, S., ... & Bradbury, P. J. (2014). Novel methods to optimize genotypic imputation for low-coverage, next-generation sequence data in crop plants. *The Plant Genome*, *7*(3), plantgenome2014-05.

Tam, V., Patel, N., Turcotte, M., Bossé, Y., Paré, G., & Meyre, D. (2019). Benefits and limitations of genome-wide association studies. *Nature Reviews Genetics*, *20*(8), 467-484.

Team, R. C. (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2012.

Thyssen, G. N., Naoumkina, M., McCarty, J. C., Jenkins, J. N., Florane, C., Li, P., & Fang, D. D. (2018). The P450 gene CYP749A16 is required for tolerance to the sulfonylurea herbicide trifloxysulfuron sodium in cotton (Gossypium hirsutum L.). *BMC plant biology*, *18*(1), 1-8.

Turner, S. D. (2018). qqman: an R package for visualizing GWAS results using QQ and manhattan plots. J. Open Source Softw. 3: 731.

USDA NASS. (2022). Quick Stats. US Department of Agriculture. National Agricultural Statistics Service. https://quickstats.nass.usda.gov/

Van Heerwaarden, J., Doebley, J., Briggs, W. H., Glaubitz, J. C., Goodman, M. M., de Jesus Sanchez Gonzalez, J., & Ross-Ibarra, J. (2011). Genetic signals of origin, spread, and introgression in a large sample of maize landraces. *Proceedings of the National Academy of Sciences*, *108*(3), 1088-1092.

Vázquez-Carrillo, M. G., Santiago-Ramos, D., & de Dios Figueroa-Cárdenas, J. (2019). Kernel properties and popping potential of Chapalote, a Mexican ancient native maize. *Journal of Cereal Science*, *86*, 69-76.

Vigouroux, Y., Glaubitz, J. C., Matsuoka, Y., Goodman, M. M., Sánchez G, J., & Doebley, J. (2008). Population structure and genetic diversity of New World maize races assessed by DNA microsatellites. *American journal of botany*, *95*(10), 1240-1253.

Vittorazzi, C., Junior, A. A., Guimarães, A. G., Silva, F. H. L., Pena, G. F., Daher, R. F., ... & Lima, V. J. (2018). Evaluation of genetic variability to form heterotic groups in popcorn. *Genet. Mol. Res*, *17*, 18083.

Wallace, J. G., Larsson, S. J., & Buckler, E. S. (2014). Entering the second century of maize quantitative genetics. *Heredity*, *112*(1), 30-38.

Wang, L., Beissinger, T. M., Lorant, A., Ross-Ibarra, C., Ross-Ibarra, J., & Hufford, M. B. (2017). The interplay of demography and selection during maize domestication and expansion. *Genome biology*, *18*(1), 1-13.

White, M. R., Mikel, M. A., de Leon, N., & Kaeppler, S. M. (2020). Diversity and heterotic patterns in North American proprietary dent maize germplasm. *Crop Science*, *60*(1), 100-114.

Wickham, H., Chang, W., & Wickham, M. H. (2016). Package 'ggplot2'. *Create elegant data visualisations using the grammar of graphics. Version*, *2*(1), 1-189.

Yin, L., Zhang, H., Tang, Z., Xu, J., Yin, D., Zhang, Z., ... & Liu, X. (2021). rMVP: a memory-efficient, visualization-enhanced, and parallel-accelerated tool for genome-wide association study. *Genomics, proteomics & bioinformatics*, *19*(4), 619-628.

Yu, D., Wang, H., Gu, W., Qin, T., Sun, P., Lu, Y., ... & Zheng, H. (2021). Genetic diversity and population structure of popcorn germplasm resources using genome-wide SNPs through genotyping-by-sequencing. *Genetic Resources and Crop Evolution*, *68*(6), 2379-2389.

Yu, G., Smith, D. K., Zhu, H., Guan, Y., & Lam, T. T. Y. (2017). ggtree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods in Ecology and Evolution*, *8*(1), 28-36.

Yuan, J. S., Tranel, P. J., & Stewart Jr, C. N. (2007). Non-target-site herbicide resistance: a family business. *Trends in plant science*, *12*(1), 6-13.

Ziegler, Kenneth E. "Popcorn." *Specialty corns*. CRC Press, 2000. 211-246.