Standardized genome-wide function prediction enables comparative functional genomics: a new application area for Gene Ontologies in plants

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Background Genome-wide gene function annotations are useful for hypothesis generation and for prioritizing candidate genes responsible for phenotypes of interest. We functionally annotated the genes of 18 crop plant genomes across 14 species using the GOMAP pipeline.

Results By comparison to existing GO annotation datasets available for a subset of these genomes, GOMAP-generated datasets cover more genes, assign more GO terms, and produce datasets similar in quality (based on precision and recall metrics using existing gold standards as the basis for comparison). From there, we sought to determine whether the datasets could be used in tandem to carry out comparative functional genomics analyses. As a test of the idea and a proof of concept, we created parsimony and distance-based dendrograms of relatedness based on functions for all 18 genomes. These dendrograms were compared to well-established species-level phylogenies to determine whether trees derived through the analysis of gene function agree with known evolutionary histories, which they largely do. Where discrepancies were observed, we determined branch support based on jack-knifing then removed individual annotation sets by genome to identify the annotation sets causing errant relationships.

Conclusions Based on the results of these analyses, it is clear that for genome assembly and annotation products of similar quality, GOMAP-derived functional annotations used together across species do retain sufficient biological signal to recover known phylogenetic relationships, indicating that comparative functional genomics across species based on GO data hold promise as a tool for generating novel hypotheses about gene function and traits.

Keywords: Gene function; ontology; plants; comparative genomics; functional genomics

I. BACKGROUND

Phenotypes and traits that have long been the focus of interest for biological investigations. Phenotypes are a result of a complex interplay between functions of genes and environmental cues. In an effort to organize and model gene functions, various systems of classification have been developed including systems like KEGG (the Kyoto Encyclopedia of Gens and Genomes), which is focused on protein function including gene activities superimposed on metabolic pathways. Other such systems include the various Cyc databases, MapMan, and the Gene Ontologies (GO), a vocabulary of gene functions organized as a directed acyclic graph, which makes it innately tractable for computational analysis [1, 2, 3, 4].

GO-based gene function annotation involves the association of GO terms to individual genes. Functions may be assigned to genes based on different types of evidence for the association. For example, functional predictions

can be inferred from experiments (EXP), inferred from expression pattern (IEP), and more [5]. Computational pipelines often are used to generate functional predictions for newly sequenced genomes, where the genome is first sequenced and assembled, then gene structures (gene models) are predicted, then functions are associated with those gene predictions. Genome-wide gene function prediction datasets are frequently used to analyze gene expression studies, to prioritize candidate genes linked to a phenotype of interest, to design experiments aimed at characterizing functions of genes, and more [6, 7, 8]. Clearly, how well a gene function prediction set models reality is strongly influenced by the how complete and correct the underlying genome assembly and gene structure annotations are coupled with how well the software used to predict functions performs.

GOMAP (the Gene Ontology Meta Annotator for Plants) is a gene function prediction system for plants that generates high-coverage and reproducible functional annotations [9]. The system employs multiple functional prediction approaches, including sequence similarity, protein domain presence, and mixed-method pipelines developed to compete in the Critical Assessment of Function Annotation (CAFA) Challenge [10], a community challenge that has advanced the performance of gene function prediction pipelines over the course of five organized competitions [11].

We previously annotated gene functions for the maize

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B73 genome and demonstrated that GOMAP's predicted functions were closer to curated gene-term associations from the literature than those of other community functional annotation datasets ([12]). Using the newly containerized GOMAP system ([9]), we report the functional annotation of 18 plant genomes across the 14 crop plant species shown in Table I.

We were curious to find out whether the datasets could be used together as a set to reveal biologically relevant and interesting perspectives. As a first step in that direction, we describe here a method by which we used gene function annotations to generate dendrograms of genome-level similarity in function (see Figure 1 for process overview). This idea is similar to that of Zhu et al., who determined the evolutionary relationships among microorganisms based on whole-genome functional similarity [13]. Here we expand on that approach, analyzing genome-wide GO assignments to generate parsimony and distance-based dendrograms. We compared these with well-established species phylogenies (Figure 2) to determine whether trees derived from gene function agree with evolutionary histories and to better consider whether these datasets could be used together for comparative functional genomics analyses.

II. RESULTS OF ANALYSES

A. Overview

Figure 1 gives an overview of the general workflow of analyses. In brief, gene function annotation sets were created and compiled for each genome. For those with existing annotation sets available, the datasets were compared. From there, matrices that included genomes as rows and terms as columns were generated. These were used directly to build parsimony trees or to create distance matrices for neighbor-joining tree construction [32, 33, 34]. In subsequent analyses, jackknifing was used to remove terms (columns) or to remove genomes (rows) to map the source of signal for treebuilding results [35].

B. Functional Annotation Sets Produced

Table II shows quantitative attributes of each of the annotation sets. In summary, GOMAP covers all annotated genomes with at least one annotation per gene, and provides between 3.8 and 12.1 times as many annotations as Gramene or Phytozome [36, 37].

Quality evaluation of gene function predictions is not trivial and is approached by different research groups in different ways. Most often datasets are assessed by comparing the set of predicted functions for a given gene to a *Gold Standard* consisting of annotations that are assumed to be correct. This assumption of correctness can be based on any number of criteria. Here we used as our Gold Standard dataset all annotations present in

Gramene that had a non-IEA (non-Inferred by Electronic Annotation) evidence code. This enabled us to assess ten of the eighteen annotation sets, as shown in Table II. It is perhaps noteworthy that the IEA and non-IEA annotation sets from Gramene frequently contain overlaps, indicating that some of the predicted annotations were manually confirmed afterwards by a curator and that in such cases, a new annotation was asserted with the new evidence code rather than simply upgrading the evidence code from IEA to some other code, thus preserving the IEA annotations in Gramene that are produced by the Ensembl analysis pipeline [38].

There are many different metrics that have been used to evaluate the quality of predicted functional annotations. For the maize B73 GOMAP [12] annotation assessment, we used a modified version of the hierarchical evaluation metrics originally introduced in [39] because they were simple, clear, and part of an earlier attempt at unifying and standardizing GO annotation comparisons [40]. In the meantime, [41] published an approach for evaluating different metrics showing differences among the robustness of different approaches to quality assessment. Here we used the SimGIC2 and Term-centric Area Under Precision-Recall Curve (TC-AUCPCR) metrics recommended by [41]. We also evaluated with the $F_{\max}$ metric, because it is very widely-used (e.g., by [10]), but according to [41] it is actually a flawed metric. Results of the quality assessments for the 10 genomes where a Gold Standard was available are shown in Table III and Figure S1. While evaluation values differ between metrics and the scores are not directly comparable, a few consistent patterns emerge: GOMAP annotations are almost always better than Gramene and Phytozome annotations in the Cellular Component and Molecular Function aspect, with the only three exceptions being the Molecular Function aspect for *Triticum aestivum* using the TC-AUCPCR and the $F_{\max}$ metric and the Cellular Component aspect for *Medicago truncatula* A17 using the $F_{\max}$ metric. Conversely, GOMAP predictions achieve consistently lower quality scores in the Biological Process aspect with the exception of *Brachypodium distachyon*, *Oryza sativa*, and *Sorghum bicolor* with the TC-AUCPCR metric. Generally, annotations that are better in one aspect are also better in the other two aspects (there are few cross-overs in Figure 9), but the ranking of annotations does not necessarily hold across metrics. The Phytozome annotation for *Oryza sativa* is an outlier in terms of its comparative quality, potentially because it is based on a modified structural annotations that differ substantially from the Gold Standard and the other annotations under comparison.

C. Phylogenetic Tree Analyses

With the comparative quality of gene function predictions in hand, we approached the question of whether the datasets could be used together for comparative func-

Table I: Functional annotation sets generated by GOMAP. More information about each dataset including the source of the input to GOMAP can be found at the respective DOI.

Species	Germplasm/Line	Assembly/Annotation	Dataset DOI
<i>Arachis hypogaea</i>	Tifrunner	Arachis hypogaea assembly 1.0	[14]
<i>Brachypodium distachyon</i>	Bd21	Bd21.v3.1.r1	[15]
<i>Cannabis sativa</i>	Hemp	NCBI Cannabis sativa GCA_900626175.1	[16]
<i>Glycine max</i>	Williams 82	Joint Genome Institute (JGI) Wm82.a4.v1	[17]
<i>Gossypium raimondii</i>	Cotton D	Gossypium raimondii JGI v2.1	[18]
<i>Hordeum vulgare</i>	–	IBSC_PGSB.r1	[19]
<i>Medicago truncatula</i>	R108_HM340	R108: v1.0	[20]
<i>Medicago truncatula</i>	A17_HM341	Mt4.0v2	[21]
<i>Oryza sativa</i>	japonica	IRGSP 1.0	[22]
<i>Phaseolus vulgaris</i>	G19833	DOE-JGI and USDA-NIFA annotation 2.0	[23]
<i>Pinus lambertiana</i>	Sugar Pine	TreeGenesDB sugar pine assembly v1.5	[24]
<i>Sorghum bicolor</i>	BTx623	BTx623.v3.0.1.r1	[25]
<i>Triticum aestivum</i>	Chinese Spring	IWGSC RefSeq 1.1	[26]
<i>Vigna unguiculata</i>	IT97K-499-35	JGI annotation v1.1	[27]
<i>Zea mays</i> *	Mo17	Zm-Mo17-REFERENCE-CAU-1.0	[28]
<i>Zea mays</i> *	PH207	Zm-PH207-REFERENCE_NS-UIUC_UMN-1.0	[29]
<i>Zea mays</i> *	W22	Zm-W22-REFERENCE-NRGENE-2.0 Zm00004b.1	[30]
<i>Zea mays</i> *	B73	RefGen_V4 Zm00001d.2	[31]

Latest overview at <https://dill-picl.org/projects/gomap/gomap-datasets/>

* Previously published in [9].

tional analysis across all genomes. As a step in that direction, we began work toward understanding the degree to which trees built based on gene functions would agree with known, well-documented evolutionary relatedness patterns. We constructed and neighbor-joining and parsimony trees of the 18 plant genomes, and visualized them using iTOL [42], the two tree topologies, rooted at *P. lambertiana*, were compared to one another and to the topology of the expected tree (Figure 2). For both the neighbor-joining (Figure 3a) and parsimony trees (Figure 3b), one common difference is noted: *S. bicolor* is not at the base of the *Z. mays* clade as expected, and is clustered with *B. distachyon* instead. Notable differences between the neighbor-joining and parsimony tree are the following: *C. sativa* appears at the base of the eudicots instead of *G. raimondii* in the neighbor-joining tree, while *G. raimondii* is grouped with *A. hypogaea* and *C. sativa* is grouped with *G. max* in the parsimony tree. Second, *O. sativa* was expected to be at the base of the BOP clade, but appears at the base of *Z. mays* in the neighbor-joining tree, but at the base of all angiosperms in the parsimony tree. Differences among relationships within the *Z. mays* clade constaining B73, PH207, W22, and Mo17 were disregarded given the high degree of similarity across annotation sets and the fact that these relationships are not clear given the complex nature of within-species relationships

Due to differences between the function-based dendrograms and the expected tree, jackknifing analysis was carried out by removing terms (columns in underlying datasets) to determine the degree to which the underlying

datasets support specific groupings based on functional term assignments. This analysis was carried out for both neighbor-joining and parsimony trees. First, trees were generated by omitting 5% then 10%, then 15% on up to 95% of the dataset to determine the threshold at which the tree topologies deviated from those generated using the full dataset. That threshold was at 45%; therefore we used trees generated with 40% of the data removed for reporting branch support for the topology (Figure 3). Comparing the two trees, the topologies were similar at jackknife values up to 40% but the support values for parsimony were comparatively lower. Based on this robustness for neighbor-joining treebuilding in general, we carried out all subsequent analyses using neighbor-joining treebuilding methods.

To map the source of discrepancies to specific gene annotation sets, we generated various neighbor-joining trees excluding one genome each time, an additional tree with both *Medicago* genomes excluded, and another with all *Z. mays* genomes excluded. To exemplify this, see the monocot clade in Figure 2 and the lower (monocot) clade in Figure 3a. When the neighbor-joining tree was generated, two species are misplaced: *S. bicolor* and *O. sativa*. As shown in Figure 4a, removal of *O. sativa* corrects one error (itself) but does not correct the errant grouping of *S. bicolor* with *B. distachyon*. In 4b, it is shown that the removal of *S. bicolor* corrects the errant grouping of itself and *B. distachyon*, but *O. sativa* placement remains incorrect. However, as shown in 4d, the removal of *B. distachyon* generates a tree where all relationships are in agreement with known species-level relationships. (Note

a. Workflow Overview

b. Workflow Detail

Figure 1: Data workflow schema. The workflow overview is shown in panel 'a' with steps represented as black boxes and the flow of information and processes indicated by arrows. Details are shown in panel 'b' where the upper large hatched box shows process detail for a single genome and the lower hatched box represents additional genomes for which the details of processing are identical. White boxes represent input datasets. Arrows indicate the flow of information and processes.

well: all individual annotation sets were progressively removed, not just these three shown in the example.)

With this observation in hand, we sought to determine the minimum number of genomes that could be removed to create a tree that matched the expected tree topology. The removal of the three genomes was required to generate function-based trees consistent with known phylogenetic relationships. They are *C. sativa*, *G. max*, and *B. distachyon* (Figure 5). Jackknifing analysis was also carried out for this dataset with support shown. Branch support is generally higher than that for the full dataset (i.e., branch support is higher in Figure 5 than in Figure 3a).

D. Potential Causes of Unexpected Groupings

As a first step in investigating whether and how the comparative quality of assemblies and annotation sets underlying the predicted gene function datasets could explain discrepancies between known evolutionary relationships and those resulting from comparative analysis of genome-wide gene function predictions, we assessed the quality of each genome assembly and structural annotation set using GenomeQC [43]. Assembly quality (Table

Figure 2: Phylogenetic relationships among species. Cladogram is rooted by the gymnosperm *Pinus lambertiana* (black). Among angiosperms, eudicots clades include Malvaceae (blue), Rosaceae (magenta), Dalbergieae (grey), and NPAAA (green). Monocots include members of the BOP (purple) and PACMAD-Panicoideae (red) clades.

Figure 3: Neighbor-joining and parsimony trees. Phylograms are colored and rooted as described in Figure 1. For both neighbor-joining (a) and parsimony (b), node values represent the jackknifing support values derived by removing 40% of GO terms in the dataset. Dashed lines mark deviations from known phylogenetic relationships. Tree scales are shown above each, with NJ showing distances and parsimony showing changes in character state.

IV), structural annotation measures of quality (Table V), and proportion of single-copy BUSCOS (short for benchmarking universal single-copy genes; [44]) (Figures 8 and 9, respectively) can be compared across assembly and annotation products. As shown in Table IV, among the genomes available via GenomeQC, *G. max* and *B. distachyon* have comparatively low values for percent of the genome that is useful (as calculated based on scaffold number and scaffold size relative to published genome sizes). In addition, as shown in Figure 7, the number of gene models <200 base pairs in length is comparatively high for *G. max*. Compounding these issues, for *G. max*, structural annotations for BUSCOS are duplicated for many *G. max* genes, which is to be expected for a hexaploid genome. Because the method used for tree construction collapses all GO terms to presence or absence rather than taking the number of instances of a particular term into account, it is unclear how the duplicate nature of the genome affects these analyses.

In the case of *C. sativa*, the fact that the line sequenced is not inbred may account for misplacement in the function-based dendrograms. This means that to generate an assembly, there are likely regions where alleles are not aligned, which would inflate the length of the assembly. Indeed, the comparatively low-quality assembly for *Cannabis* genome has been noted by others [45], and our preliminary investigations indicate that the assembly length is in fact longer than expected based on C-values for genome sizes reported previously [46]. In addition, we found 184 GO terms missing from *C. sativa* that were present in the rest of the eudicots, and 93 additional terms that were only in *C. sativa* but none of the other eudicots in our study. This finding could con-

firm the poor quality of the *C. sativa* genome used in our dataset. More efforts to determine in detail the causes of these discrepancies are currently underway.

III. DISCUSSION

In this study, we used the GOMAP pipeline to produce whole-genome GO annotations for eighteen genome assembly and annotation sets from fourteen plant species [9]. Assessments of the number of terms predicted as well as the quality of predictions (based on F-score) indicate that GOMAP functional prediction datasets cover more genes, produce more predictions per gene, and are of similar quality to smaller prediction datasets produced by other systems, thus supporting the notion that these high-coverage datasets are a useful addition for researchers who are interested in genome-level analyses, including efforts aimed at prioritizing candidate genes for downstream analyses. Given that we can now produce high-quality, whole genome functional annotations for plants in a straightforward way, we intend to produce more of these over time (indeed we recently annotated *Vitis vinifera* Pinot Noir grape [47], *Brassica rapa* (doi in process), *Musa acuminata* [48], *Theobroma cacao* [49], *Solanum lycopersicum* [50], and *Solanum pennellii* [51]).

With eighteen genome functional annotations in hand, we sought to determine whether and how researchers could use multispecies GO annotation datasets to perform comparative functional genomics analyses. As a first step in that direction and a proof of concept, we adapted phylogenetic tree-building methods to use the gene function terms assigned to genes represented in

Figure 4: Restoring monocot relationships. Phylograms are colored and rooted as described in Figure 1. Dashed lines mark deviations from known phylogenetic relationships. Monocot topology changes with removal of a single species: a) *O. sativa*, b) *S. bicolor*, and c) *B. distachyon*. Tree scale is shown above.

genomes to build dendrograms of functional relatedness and hypothesized that if the functions were comparable across species, the resulting trees would closely match evolutionary relationships. To our delight and surprise, the neighbor-joining and parsimony trees (Figure 3) did resemble known phylogenies, but were not exact matches to broadly accepted phylogenetic relationships.

After removing the minimum number of genomes that resulted in restoration of the expected evolutionary relationships, we found that the individual species that may be responsible for the discrepancies observed in Figure 3 were *C. sativa*, *G. max*, and *B. distachyon*. We hypothesize in a general way that the following reasons could account for these errant relationships:

1) Quality of sequencing and coverage assembly: genomes of similarly high sequence coverage that have excellent gene calling would be anticipated to create the

Figure 5: Restoring known phylogenetic relationships to the NJ tree via removal of a minimal number of species. Phylograms are colored and rooted as described in Figure 1. Node values represent the jackknifing support values derived by removing 40% of GO terms in the dataset. 3 genomes have been removed: *C. sativa*, *G. max*, and *B. distachyon*. Tree scale is shown above.

best source for functional annotation. Genomes of comparatively lower, or different, character would be anticipated to mislead treebuilding and other comparative genomics approaches.

2) Reference guided assembly: if one genome is used to guide the assembly and annotation of another genome, some similarities may result naturally due to the inheritance of information.

3) Shared selected or natural traits: species that have been selected for, e.g., oilseeds may share genes involved in synthesis of various oils. Other shared traits would be anticipated to cause similarities for species with those shared traits.

For the species noted in this paper as misleading treebuilding based on whole-genome gene function, we find that the most likely cause of discrepancies between phylogenetic trees and those inferred from gene functions is the simplest: differences in quality of input sequence assemblies and gene structure annotations. The particular differences are our current focus for investigation and are anticipated to result in updates and additions to this manuscript.

In conclusion, we have demonstrated that the GOMAP system produces datasets can be used together for comparative functional genomics analyses if the datasets are derived from comparably high-quality assemblies and gene annotations. We look forward not only to developing systems to support comparative functional genomics tools, but also to seeing the tools other research groups will develop to approach these datasets for use to formulate useful comparative functional genomics hypotheses.

Figure 6: Assembly BUSCO plot generated using GenomeQC. Genomes analyzed are shown across the X-axis, and are ordered to match the occurrence of species shown in Figure 2. Percentage of BUSCO genes across four gene categories are stacked, with each adding up to 100 percent (Y-axis). Complete and single-copy genes are shown in grey, complete and duplicated copies in orange, fragmented copies in blue, and missing are shown copies in green.

IV. METHODS

A. Acquiring Input Datasets

For each of the 18 genomes listed in Table I, information on how to access input annotation products are listed in the DOI shown. For each, one representative translated peptide sequence per protein coding gene was selected and used as the input for GOMAP. Unless the authors of the genome provided a set of representative sequences designated as canonical, we chose the longest translated peptide sequence as the representative for each gene model. Non-IUPAC characters and trailing asterisks (*) were removed from the sequences, and headers were simplified to contain only non-special characters. The corresponding script for each dataset can be found at the respective DOI that required these changes. Based on this input, GOMAP yielded a functional annotation set spanning all protein-coding genes in the genome. Using the Gene Ontology version releases/2020-10-09, this functional annotation set was cleaned up by removing

duplicates, annotations with modifiers, and obsolete GO terms. Any terms containing alternative identifiers were merged to their respective main identifier, which uncovered a few additional duplicates, which were also removed. Table SI shows the number of annotations removed from each dataset produced.

To compare the quality of GOMAP predictions to currently available functional predictions from Gramene and Phytozome, we downloaded IEA annotations from Gramene (version 63, [36], <https://www.gramene.org/>) and Phytozome (version 12, [37], <https://phytozome.jgi.doe.gov/>) for each species with functional annotations of the same genome version. These datasets were cleansed of duplications and redundancies. Similarly cleaned non-IEA annotations from Gramene served as the Gold Standard wherever they were available. More detailed information on how we accessed these datasets can be found at https://github.com/Dill-PICL/GOMAP-Paper-2019.1/blob/master/data/go_annotation_sets/README.md.

Figure 7: Annotation BUSCO plot generated using GenomeQC. Genomes analyzed are shown across the X-axis, and are ordered to match the occurrence of species shown in Figure 2. Percentage of BUSCO genes across four gene categories are stacked, with each adding up to 100 percent (Y-axis). Complete and single-copy genes are shown in grey, complete and duplicated copies in orange, fragmented copies in blue, and missing are shown copies in green.

B. Quantitative and Qualitative Evaluation

The number of annotations in each cleansed dataset was determined and related to the number of protein coding genes (based on transcripts in the input FASTA file). This was done for separately for each GO aspect as well as in total (see Table II).

The ADS software version published in [41] is available from <https://bitbucket.org/plyusnin/ads/>. We used version b6309cb (also included in our code as a submodule) to calculate SimGIC2, TC-AUCPCR, and $F_{\max}$ quality scores. To provide the information content required for the SimGIC2 metric, the Arabidopsis GOA from https://www.ebi.ac.uk/GOA/arabidopsis_release was used in version 2021-02-16.

C. Cladogram Construction

For clustering, we first collected all GO terms annotated to any gene in each genome into a list and re-

moved the duplicates, yielding a one-dimensional set of GO terms for each genome (T). Next, we added all ancestor terms for each term in this set T and once again removed the duplicates, yielding a set S . These sets with added ancestors served as a starting point of our tree-building analyses: pairwise distances between the genomes were calculated using the Jaccard distance as a metric of the dissimilarity between any two sets a and b .

$$d_{ab} = 1 - \frac{|S_a \cap S_b|}{|S_a \cup S_b|} \quad (1)$$

Then a neighbor-joining tree was constructed on the pairwise distance matrix using PHYLIP [32]. Additionally, term sets S of all genomes were combined into a binary matrix (with rows corresponding to genomes and columns corresponding to GO terms, values of 0 or 1 indicating whether a term is present or absent in the given set). PHYLIP pars was used to construct a parsimony tree from this binary matrix.

P. lambertiana, a gymnosperm, was included in the dataset as an outgroup to the angiosperms to separate

between the monocot and eudicot plants. iTOL [52] was used to visualize the trees using their Newick format, and root them at *P. lambertiana*. Moreover, a cladogram representing the known phylogeny of the included taxa was created by hand based on known evolutionary relationships [53, 54, 55, 56, 57]. This was used to compare the generated phylogenetic relationship based on functional similarity with the evolutionary relationships of the plant genomes.

Jackknifing analysis was carried out for both parsimony and neighbor-joining trees to assess the support for each clade based on the proportion of jackknife trees showing the same clade. To this end, 40% of the terms in *T* were randomly removed, ancestors of the remaining terms were added and trees constructed as above. The majority rule consensus tree of 100 individual trees was calculated with the jackknife values represented on each branch. The tree was then visualized using iTOL using its Newick format, and rooted again at *P. lambertiana*.

D. Genome Quality Evaluation

Genome size was estimated from the C-values obtained from the Plant DNA C-values data resource from the Kew Database (<https://cvalues.science.kew.org/>). The mean C-value for a given species was used for calculating genome size estimates in base pairs (bp) using the method of [58]. In brief,

$$\text{Genome size (bp)} = \text{C-value (pg)} * 0.978 \times 10^9 \frac{\text{bp}}{\text{pg}}$$

The estimated genome size (listed in Table IV) was used as an input for GenomeQC (<https://genomeqc.maizegdb.org/>) [43] to calculate quality metrics. For genomes that were too large to submit through the GenomeQC webtool or had missing exon information, modified scripts of those found in GitHub of GenomeQC (<https://github.com/HuffordLab/GenomeQC>, commit e6140ee) were applied to calculate the assembly and structural annotation metrics in Table IV and V. BUSCO version 5.0.0 [59] was used to calculate the assembly and annotation BUSCO scores, shown in Figures 6 and 7. The input for assembly BUSCO scores were chromosome sequences, whereas inputs were transcript/mRNA/CDS sequences for the annotation BUSCO scores. For the lineage parameter, the lineage datasets used were as follows: Eudicots for *C. sativa* and *G. raimondii*, Fabales for *A. hypogaea*, *M. truncatula* A17 and R108, *P. vulgaris*, *G. max*, and *V. unguiculata*, and Poales for *B. distachyon*, *O. sativa*, *T. aestivum*, *H. vulgare*, *S. bicolor* and *Z. mays* B73, Mo17, W22 and PH207.

V. AVAILABILITY OF SOURCE CODE AND SUPPORTING DATA

All used data and source code are freely available at <https://github.com/Dill-PICL/GOMAP-Paper-2019>. 1 under the terms of the MIT license. All software requirements and dependencies are packaged into a Singularity container so no other setup is required. We will provide a DOI through Zenodo for the final version of the manuscript after reviews and corrections are incorporated.

An up-to-date list of all available annotation sets can be found at <https://dill-picl.org/projects/gomap/gomap-datasets/>.

VI. DECLARATIONS

A. Competing Interests

The author(s) declare that they have no competing interests.

B. Funding

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C. Author's Contributions

LF, DP, DFY, KC, HAD, PJ, DCS, HV, and KW generated annotations for plants as described in this paper. DP and CJLD co-conceived the idea for phylogenetic analysis. LF worked with DP to create dendrograms and compare those to phylogenetic trees. LF carried out assembly and annotation metric comparisons. LF, DP, and CJLD wrote the manuscript. All authors read, offered suggestions to improve, and approved the final copy of the manuscript.

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VIII. AUTHORS' INFORMATION

KW created the GOMAP system during his time as a graduate student at Iowa State University. LF, DP, CFY, KC, HV, and PJ are currently graduate students. HAD and DCS are undergraduate students. Each graduate and undergraduate student annotated at least one genome over the course of a research rotation lasting no more than one semester. CJLD coordinated research activities and manuscript preparation.

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Table II: Quantitative metrics of the cleaned functional annotation sets. CC, MF, BP, and A refer to the aspects of the Gene Ontology: Cellular Component, Molecular Function, Biological Process, and Any/All. GOMAP covers all genomes with at least one annotation per gene and provides substantially more annotations than Gramene63 or Phytozome, especially in the BP aspect.

Genome	Genes	Dataset	Genes Annotated[%] ^a				Annotations ^b				Median Ann. per G. ^c			
			CC	MF	BP	A	CC	MF	BP	A	CC	MF	BP	A
<i>Arachis hypogaea</i>	67,124	GOMAP	85.85	84.68	100.00	100.00	150,525	132,144	493,145	775,814	2	2	6	10
<i>Brachypodium distachyon</i>	34,310	GOMAP	81.33	85.35	100.00	100.00	74,172	69,213	255,397	398,782	2	2	6	10
		GoldStandard	21.54	19.53	18.20	26.66	10,985	10,436	11,120	32,673	1	1	1	3
		Gramene63-IEA	33.12	49.29	38.29	63.60	21,658	36,372	23,899	82,026	1	1	1	3
		Phytozome12	10.25	37.21	26.86	43.11	4,186	18,597	11,070	34,060	0	1	1	2
<i>Cannabis sativa</i>	33,677	GOMAP	94.22	95.48	100.00	100.00	85,755	73,614	262,741	422,110	2	2	6	11
<i>Glycine max</i>	52,872	GOMAP	86.95	88.92	100.00	100.00	126,470	113,068	416,989	656,527	2	2	6	11
<i>Gossypium raimondii</i>	37,505	GOMAP	93.00	92.37	100.00	100.00	95,419	84,910	307,470	487,799	2	2	6	11
<i>Hordeum vulgare</i>	39,734	GOMAP	88.57	91.76	100.00	100.00	86,489	79,727	272,420	438,636	2	2	5	10
		GoldStandard	28.23	26.30	23.43	35.64	15,734	15,391	15,267	46,414	1	1	1	3
		Gramene63-IEA	36.19	50.90	41.71	65.03	29,826	44,789	29,425	104,178	1	1	1	3
		Phytozome12	8.87	36.05	25.83	41.07	5,315	25,950	15,576	47,098	0	1	1	2
<i>Medicago truncatula</i> A17	50,444	GOMAP	83.79	86.69	100.00	100.00	104,902	99,155	363,608	567,665	2	2	6	10
		GoldStandard	25.45	23.26	21.51	32.12	17,938	18,416	18,461	54,827	1	1	1	3
		Gramene63-IEA	34.25	50.84	40.26	66.14	32,753	63,470	40,441	137,001	1	1	1	3
		Phytozome12	8.87	36.05	25.83	41.07	5,315	25,950	15,576	47,098	0	1	1	2
<i>Medicago truncatula</i> R108	55,706	GOMAP	72.10	90.14	100.00	100.00	108,388	107,499	381,831	597,718	1	2	5	9
<i>Oryza sativa</i>	35,825	GOMAP	79.78	83.31	100.00	100.00	71,306	64,150	248,304	383,760	2	2	6	9
		GoldStandard	29.95	27.29	25.33	37.57	15,492	15,176	16,536	47,339	1	1	1	3
		Gramene63-IEA	32.21	45.83	36.75	60.13	21,935	37,425	24,255	83,645	1	1	1	3
		Phytozome12	10.31	40.10	29.18	46.09	4,361	20,842	12,451	37,884	0	1	1	2
<i>Phaseolus vulgaris</i>	27,433	GOMAP	94.48	93.06	100.00	100.00	70,987	64,022	229,230	364,239	2	2	6	11
<i>Pinus lambertiana</i>	31,007	GOMAP	92.67	95.91	100.00	100.00	71,247	68,315	212,248	351,810	2	2	5	10
<i>Sorghum bicolor</i>	34,129	GOMAP	82.44	85.98	100.00	100.00	75,145	69,659	259,004	403,808	2	2	6	10
		GoldStandard	34.48	32.91	30.90	42.84	16,837	17,614	17,850	52,593	1	1	1	3
		Gramene63-IEA	35.91	52.11	42.36	67.41	23,608	39,418	27,074	90,313	1	1	1	3
		Phytozome12	10.54	39.19	27.90	45.10	4,246	19,724	11,432	35,599	0	1	1	2
<i>Triticum aestivum</i>	107,891	GOMAP	88.53	90.98	100.00	100.00	259,318	217,467	785,051	1,261,836	2	2	6	10
		GoldStandard	2.98	2.78	2.56	3.82	4,727	4,512	4,793	14,035	1	1	1	3
		Gramene63-IEA	29.12	58.62	38.72	70.41	47,595	111,889	62,977	222,721	0	1	1	2
<i>Vigna unguiculata</i>	29,773	GOMAP	91.21	91.08	100.00	100.00	74,791	67,734	242,847	385,372	2	2	6	11
		Phytozome12	13.91	45.68	34.14	53.06	5,107	19,962	12,209	37,534	0	1	1	2
<i>Zea mays</i> B73.v4	39,324	GOMAP	93.16	94.92	100.00	100.00	87,648	81,665	278,305	447,618	2	2	6	10
		GoldStandard	37.92	34.78	32.67	46.85	22,531	21,292	23,153	67,285	1	1	1	3
		Gramene63-IEA	39.16	58.16	48.21	73.87	30,189	53,748	35,276	119,273	1	1	1	3
<i>Zea mays</i> Mo17	38,620	GOMAP	86.98	90.87	100.00	100.00	86,074	78,650	277,395	442,119	2	2	6	10
		GoldStandard	27.56	25.20	23.73	33.98	16,128	15,384	16,489	48,220	1	1	1	3
<i>Zea mays</i> PH207	40,557	GOMAP	86.55	90.61	100.00	100.00	88,962	84,910	288,208	462,080	2	2	6	10
		GoldStandard	28.18	25.82	24.26	34.66	17,370	16,580	17,791	51,984	1	1	1	3
<i>Zea mays</i> W22	40,690	GOMAP	90.77	92.58	100.00	100.00	93,622	84,450	289,364	467,436	2	2	6	10
		GoldStandard	25.40	23.15	21.80	31.29	15,518	14,818	15,850	46,402	1	1	1	3

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^a How many genes in the genome have at least one GO term from the CC, MF, BP aspect annotated to them? A = How many at least one from any aspect? (A = CC ∪ MF ∪ BP)

^b How many annotations in the CC, MF, and BP aspect does this dataset contain? A = How many in total? A = CC + MF + BP

^c Take a typical gene that is present in the annotation set. How many annotations does it have in each aspect? A = How many in total? Please note that A ≠ CC + MF + BP

Table III: Qualitative metrics of functional annotation sets predicted by GOMAP, Gramene, and Phytozome.

Genome	Dataset	SimGIC2			TC-AUCPCR			Fmax		
		CC	MF	BP	CC	MF	BP	CC	MF	BP
<i>Brachypodium distachyon</i>	GOMAP	0.404149	0.464127	0.223830	0.233442	0.230701	0.118526	0.741361	0.740897	0.526881
	Gramene63-IEA	0.317801	0.420859	0.349406	0.129163	0.192507	0.111361	0.691016	0.738542	0.650325
	Phytozome12	0.370264	0.370521	0.352206	0.112582	0.136832	0.085628	0.717759	0.697076	0.660603
<i>Hordeum vulgare</i>	GOMAP	0.400087	0.470012	0.238177	0.237231	0.261399	0.130784	0.745272	0.750213	0.560096
	Gramene63-IEA	0.306119	0.426601	0.381010	0.157352	0.228797	0.136002	0.680996	0.742638	0.665696
<i>Medicago truncatula</i> A17	GOMAP	0.371795	0.451258	0.213407	0.272809	0.282650	0.139032	0.730838	0.726991	0.531406
	Gramene63-IEA	0.329600	0.437274	0.343561	0.176497	0.265887	0.133503	0.701093	0.749900	0.654297
	Phytozome12	0.358311	0.367257	0.363013	0.144247	0.170863	0.110386	0.717307	0.698429	0.661233
<i>Oryza sativa</i>	GOMAP	0.408945	0.482650	0.248207	0.298502	0.303384	0.159724	0.751121	0.757181	0.559221
	Gramene63-IEA	0.328761	0.423191	0.341193	0.167619	0.265410	0.135451	0.711309	0.738732	0.643827
	Phytozome12	0.049975	0.041007	0.044279	0.000003	0.000003	0.000002	0.470134	0.266628	0.239256
<i>Sorghum bicolor</i>	GOMAP	0.404852	0.466708	0.224011	0.316873	0.337380	0.169883	0.746540	0.742001	0.534258
	Gramene63-IEA	0.323037	0.400241	0.353135	0.177038	0.260198	0.154157	0.711107	0.712170	0.653591
	Phytozome12	0.356091	0.348264	0.340124	0.151947	0.177579	0.110483	0.715714	0.675147	0.641535
<i>Triticum aestivum</i>	GOMAP	0.410582	0.489881	0.229271	0.050762	0.030610	0.019360	0.736476	0.762420	0.533897
	Gramene63-IEA	0.362452	0.476685	0.395112	0.040992	0.043701	0.027872	0.737769	0.762059	0.670953
<i>Zea mays</i> B73.v4	GOMAP	0.417455	0.467339	0.245373	0.302761	0.290371	0.153011	0.759504	0.746870	0.564707
	Gramene63-IEA	0.303231	0.416301	0.346308	0.175735	0.250075	0.138275	0.662987	0.732860	0.647725
<i>Zea mays</i> Mo17	GOMAP	0.399521	0.464265	0.225632	0.236209	0.239598	0.125599	0.744360	0.743026	0.537489
<i>Zea mays</i> PH207	GOMAP	0.394481	0.436266	0.224226	0.221709	0.221266	0.117086	0.743111	0.718933	0.533092
<i>Zea mays</i> W22	GOMAP	0.397602	0.463499	0.223511	0.210198	0.217609	0.113262	0.743783	0.742341	0.535572

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Table IV: Assembly statistics from GenomeQC. Note well that the species and genome versions here are not guaranteed to agree with previous datasets in this analysis.

	Scaffolds	Total Scaffold Length (bp)	Total Scaffold Length / Estimated Genome Size (%)	Scaffold Sequences >=25K nt	% of Estimated Genome with Scaffold Sequences >=25K nt	%N	Estimated Genome Size (bp)	C-value (pg)	C-value Ref.
<i>A. hypogaea</i>	384	2.56E+09	91.12	2.55E+09	91.01	0.15	2806	2.87	[60]
<i>B. distachyon</i>	11	2.72E+08	87.00	2.72E+08	87.00	0.19	313	0.32	[61]
<i>C. sativa</i>	221	8.76E+08	106.72	8.75E+08	106.62	15.93	821	0.84	[46]
<i>G. max</i>	282	9.78E+08	88.62	9.77E+08	88.48	2.65	1105	1.13	[62]
<i>G. raimondii</i>	1033	7.61E+08	86.52	7.55E+08	85.77	1.75	880	0.9	[63]
<i>H. vulgare</i>	8	4.83E+09	67.45	4.83E+09	67.45	5.44	7167	7.33	[64, 65]
<i>M. truncatula</i> A17	2186	4.13E+08	89.93	4.03E+08	87.77	5.53	460	0.47	[66]
<i>M. truncatula</i> R108	909	4.02E+08	87.60	3.95E+08	86.09	0.68	460	0.47	[66]
<i>O. sativa</i>	63	3.75E+08	76.85	3.75E+08	76.74	0.03	489	0.5	[64]
<i>P. vulgaris</i>	478	5.37E+08	91.68	5.34E+08	91.12	1.05	587	0.6	[67]
<i>S. bicolor</i>	870	7.09E+08	60.43	7.05E+08	60.08	4.72	1173	1.2	[68]
<i>T. aestivum</i>	22	1.45E+10	86.00	1.45E+10	86.00	1.90	16916	17.3	[64]
<i>V. unguiculata</i>	686	5.19E+08	88.64	5.18E+08	88.38	0.00	587	0.6	[69]
<i>Z. mays</i> B73	266	2.13E+09	80.85	2.13E+09	80.85	1.44	2640	2.7	[64]
<i>Z. mays</i> Mo17	2208	2.18E+09	82.68	2.17E+09	82.06	1.61	2640	2.7	[64]
<i>Z. mays</i> PH207	43291	2.16E+09	81.67	2.09E+09	79.25	19.61	2640	2.7	[64]
<i>Z. mays</i> W22	11	2.13E+09	80.83	2.13E+09	80.83	1.90	2640	2.7	[64]

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Table V: Structural annotation table directly from GenomeQC. Note well that the species and genome versions here are not guaranteed to agree with previous datasets in this analysis.

	Gene Models	Min. Gene Length (bp)	Max. Gene Length (bp)	Avg. Gene Length (bp)	Gene Models <200 bp	Transcripts	Avg. Transcripts per Gene Model	Exons	Avg. Exons per Transcript	Avg. Exon Length (bp)
<i>A. hypogaea</i>	67128	163	342359	3972.3	48	84714	1.3	423763	4.8	296.4
<i>B. distachyon</i>	36647	90	47411	3012.5	175	47917	1.3	263865	5.5	314.1
<i>C. sativa</i>	29815	21	976063	3450	2187	34876	1.2	234157	6.6	291.9
<i>G. max</i>	52872	90	94733	4017	-	-	-	-	-	-
<i>G. raimondii</i>	37505	90	51175	3243.5	71	77267	2.1	527563	6.7	271.3
<i>H. vulgare</i>	-	-	-	-	-	248270	-	1715898	-	278.9
<i>M. truncatula</i> A17	51541	60	102191	2566.9	1985	74213	1.4	318421	4.4	313.2
<i>M. truncatula</i> R108	55706	72	62996	2232.4	2404	61019	1.1	220904	3.6	270.6
<i>O. sativa</i>	35825	72	304271	3098.7	57	42378	1.2	192499	4.5	350.3
<i>P. vulgaris</i>	27433	93	90772	3943.8	108	36995	1.3	-	-	-
<i>S. bicolor</i>	34129	96	88337	3713.2	56	47121	1.4	266301	5.6	350.9
<i>T. aestivum</i>	107891	54	124945	3488.9	686	133744	1.2	749233	5.8	303.3
<i>V. unguiculata</i>	29773	90	81066	3880.9	408	42287	1.4	-	-	-
<i>Z. mays</i> B73	39324	111	128402	4117.8	38	151959	3.9	1488381	10	281.5
<i>Z. mays</i> Mo17	38620	21	146217	4076.8	1225	46530	1.2	272323	5.9	297.1
<i>Z. mays</i> PH207	40557	111	714207	5993.3	53	40557	1.0	208149	5.1	252.1
<i>Z. mays</i> W22	40691	87	154495	4330.4	64	51717	1.3	313830	6	292.4

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Dashes (-) represent missing data.

a. Workflow Overview

b. Workflow Detail

Tree scale: 0.1

a.

Tree scale: 1000

b.

Tree scale: 0.1

a.

b.

c.

Tree scale: 0.1

Percentage of BLASTO domain

Percentage of IL-6/IL-6R complex