1 A web-based platform of nucleotide sequence alignments of plants

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18 **Abstract**

19 In recent years, a large number of nucleotide sequences have become available for plant
20 species by the advent of massive parallel sequencing. The use of genomic data has been
21 important for agriculture, food science, medicine or ecology. Despite the increasing amount of
22 data, nucleotide sequences are usually available in public databases as isolated records with
23 some descriptive information. Researchers interested in studying a wide range of specific
24 plant families are forced to do multiple searches, sequence downloads, data curation and
25 sequence alignments. In order to help researches overcoming these problems, we have built a
26 comprehensive on-line resource of curated nucleotide sequence alignments for plant research,
27 named PlantAligDB (available at <http://plantaligdb.portugene.com>). The latest release
28 incorporates 514 alignments with a total of 66,052 sequences from six important genomic
29 regions: *atpF-atpH*, *psbA-trnH*, *trnL*, *rbcL*, *matK* and ITS. The alignments represent 223
30 plant families from a variety of taxonomic groups. The users can quickly search the database,
31 download and visualize the curated alignments and phylogenetic trees using dynamic
32 browser-based applications. Different measures of genetic diversity are also available for each
33 plant family. We also provide the workflow script that allows the user to do the curation
34 process, explaining the steps involved. Overall, the PlantAligDB provides a complete, quality
35 checked and regularly updated collection of alignments that can be used in taxonomic, DNA
36 barcoding, molecular genetics, phylogenetic and evolutionary studies.

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38 **Keywords**

39 DNA sequences, Multiple sequence alignments, Plant families

40 Introduction

41 The recent development of high-throughput sequencing technologies has significantly
 42 increased the number of nucleotide sequences available in public databases (Egan *et al.* 2012;
 43 Feuillet *et al.* 2011). Complete genome sequences are now accessible in public databases
 44 (e.g., EnsemblPlants) (<https://plants.ensembl.org/index.html>) for the analysis and visualisation
 45 of genomic data for an ever-growing number of plants, such as *Beta vulgaris*, *Prunus persica*
 46 and *Citrus sinensis*, among many others. Sequences from individual genes or gene regions
 47 have also been deposit in public databases as a result of international initiatives. For instance,
 48 the DNA barcoding project has released thousands of sequences aiming at species
 49 identification and taxonomic classification of plants, mostly from the chloroplast DNA
 50 (cpDNA) protein-coding genes *rbcL* and *matK* [CBOL Plant Working Group' - (Group *et al.*
 51 2009; Hollingsworth *et al.* 2011)]. The plastid *trnL* (UAA) intron is another good example of
 52 a cpDNA region highly represented in sequence databanks (Taberlet *et al.* 2007).

53 Several web-based databases are available for plant genome sequences, usually
 54 dedicated to a single species or a genomic feature [e.g., (Lai *et al.* 2012; Meyer *et al.* 2005;
 55 Numa & Itoh 2014; Sakai *et al.* 2013)]. However, most nucleotide sequences are accessible in
 56 public databases as isolated records with simple descriptive information (taxonomy,
 57 geography, publications, etc.). For instance, the NCBI Entrez Nucleotide database
 58 (<http://www.ncbi.nlm.nih.gov>) and the BOLD - The Barcode of Life Data System
 59 (www.barcodinglife.org) (Ratnasingham & Hebert 2007) are useful repositories with
 60 descriptive information for sequence or species. The TreeBASE (<https://www.treebase.org>) is
 61 a repository of phylogenetic information with user-submitted phylogenetic trees and the data
 62 used to generate them. Nevertheless, researchers interested in studying a large number of
 63 plant families are forced to do multiple searches and sequence downloads of genetic data for
 64 their investigations. Moreover, the available sequences are not aligned and curated. The

multiple sequence alignment step is critical because it determines the accuracy of the subsequence analyses, such as phylogenetic inference, identification of conserved motifs, function prediction, etc. Building accurate sequence alignments involves many steps, including sequence file conversion, run of alignment algorithms in local computers or web servers, selection of best alignment parameters, and manual fine-tuning of the alignment. This process is laborious and requires costly computational resources, which are not always available.

We describe an on-line database (PlantAligDB, available at <http://plantaligdb.portugene.com>) with a comprehensive, automatically curated and regularly updated collection of alignments from diverse plant families (Figure 1), and respective phylogenetic inference of each alignment. The PlantAligDB is a consistent repository of curated alignments and phylogenetic trees that are generated by the same workflow. For example, the Orchidaceae family is frequently referred to as a critical group, whose species are difficult to identify. Because of their ecological importance, ongoing studies continue to be made to reduce their extinction risk and maintain their diversity (Li *et al.* 2018). The PlantAligDB can help researchers designing accurate methods for plant identification (*matK* and *rbcL* are used in DNA barcoding projects) whether by identification of conserved motives that enable the design of primers. The PlantAligDB alignments can be used as a reference database for phylogenetic studies, allowing the construction of reference phylogenetic trees of the different regions [e.g., genomic regions *atpF-atpH* (Domenech *et al.* 2014), *psbA-trnH* (Dong *et al.* 2012) and ITS (Karehed *et al.* 2008)]. Moreover, it provides useful data to understand the genetic diversity of the selected genomic regions.

Materials and Methods

90 **Data curation**

91 We retrieved all nucleotide sequences of different genomic regions from the NCBI
 92 Entrez Nucleotide database (<http://www.ncbi.nlm.nih.gov>) using the Geneious software
 93 (Drummond 2009). Different combinations of search terms (e.g. ‘*gene name*’; ‘viridiplantae’;
 94 ‘chloroplast’; ‘gene’; ‘complete’) and a maximum limit of 5000 bp as sequence length were
 95 used in searches to retrieve the largest number of sequences. The selection was done by
 96 eliminating sequences showing one or more of the following features: a) ambiguous name
 97 description; b) sequences with high number of nucleotide ambiguities (>50%); c) sequences
 98 with large stretches of the region missing (>50%). After a preliminary curation of the data, we
 99 selected five cpDNA regions and one nuclear DNA region, which were the most represented
 100 in the NCBI database, commonly used in phylogenetic studies for being relevant and
 101 informative. The six genomic regions were named according to the gene regions where they
 102 are located: *atpF-atpH* (*ATPase I subunit – ATPase III subunit*), *psbA-trnH* [*Photosystem II*
 103 *32 kDa protein – tRNA-His (GUG)*], *trnL* [*tRNA-Leu (UAA)*], *rbcL* (*rubisco large subunit*),
 104 *matK* (*maturase K*) and ITS (internal transcribed spacer). We then removed from the datasets
 105 all redundant sequences belonging to the same species and sequences without a clear species
 106 assignment. We also reverse complement the sequences that were found in the opposite
 107 direction. The sequence orientation for each region is that of the most commonly found in the
 108 NCBI database. Therefore, the orientation of the *trnL* (*UAA*), *atpF-atpH* and *rbcL* regions are
 109 the same of that used in the reference cpDNA sequence of *Nicotiana tabacum*
 110 (NC_001879.2), while the opposite orientation is used for regions *psbA-trnH* and *matK*. The
 111 target region named ITS in our database includes the internal transcribed spacer 1, 5.8S rRNA
 112 and internal transcribed spacer 2 section of the nuclear ribosomal DNA.

113 Because a high number of sequences were detected for the *trnL* (*UAA*) region (more
 114 than 50,000 hits), we used the external regions named “C” and “D” and the internal regions

named “G” and “H” by (Taberlet *et al.* 2007) as queries in the NCBI Basic Local Alignment Search Tool (BLAST; <http://blast.ncbi.nlm.nih.gov/>). The search was made against the nucleotide collection (nr/nt) of Tracheophyta (vascular plants) using the Biopython package (www.biopython.org) with an expected threshold of 1000 and a minimum word size of 16. Therefore, our database includes two datasets for the *trnL* (UAA) genomic region: the ‘*trnL* CD’ target region with a length of 577 bp in *N. tabacum*, and the ‘*trnL* GH’ with a length of 78 bp in *N. tabacum*, located inside the *trnL* CD region. All information regarding the selected target regions can be found in the *Genomic Regions* section of the PlantAligDB.

The nucleotide sequences of the six regions were organized by family according to the NCBI taxonomy and were aligned (each region and family in separated alignments) using the default parameters of the MUSCLE software (Edgar 2004) running in the Geneious software. The alignment was repeated in some families after excluding sequences that do not cover the entire region of interest and that had large stretches of nucleotide ambiguities. We only used alignments with ten or more species per family to build the PlantAligDB. Some species sequences were lost in this process filter. The neighbor joining phylogenetic tree of each region-family were calculated using Tamura-Nei model using Geneious Tree Builder. The methodology was built in Armadillo Workflow (<http://www.bioinfo.uqam.ca/armadillo/>) to automate the update process of the database. The latest release update of June 2018 incorporates 514 alignments and phylogenetic trees, from 223 plant families.

Conservation measures

The database includes two measures of sequence conservation for each alignment: *percentage of identical sites* (PIS), calculated by dividing the number of identical positions in the alignment for an oligonucleotide by its length and the *percentage of pairwise identity* (PPI), calculated by counting the average number of pairwise matches across the positions of

the alignment, divided by the total number of pairwise comparisons.

Results and Discussion

Database organization

Basic structure

The PlantAligDB database is divided in nine sections (Figure 1): 1) *Home*, provides a brief description of what can be done in the database; 2) *Genomic regions*, describes the regions used in the database; 3) *Taxonomic groups*, the table containing the plant family/region alignments and phylogenetic trees; 4) *BLAST*, search the database using a query sequence by means of the BLASTN algorithm (Altschul *et al.* 1990); 5) *Genetic diversity*, describes the percentage of identical sites (PIS) and pairwise identity values (PPI) for each alignment; 6) *Download*, provides hyperlinks to download the curated alignments; 7) *Tutorials*, contains information about how the database was built, and how to use it; 8) *Citations*; 9) *Contacts*.

Taxonomic groups

The database is being regularly updated by our team and currently includes 514 alignments and phylogenetic trees from seven target regions: *atpF-atpH*, *psbA-trnH*, *trnL* CD, *trnL* GH, *rbcL*, *matK* and ITS (Table 1). Sequence alignments are provided for 223 different plant families. Currently, the *trnL* GH region has the largest number of sequences (n = 34,674). The *Fabaceae* family has the largest number of aligned species in a target region, with 2599 sequences for the *trnL* GH region. When considering all regions together, the *Fabaceae* (n = 4714), *Poaceae* (n = 4494) and *Asteraceae* (n = 4459) families are those with

the highest number of sequences. The alignments for each plant family can be accessed through a dynamic table in the *Taxonomic groups* section of the database by following a hyperlink with the number of species included in each alignment (http://plantaligdb.portugene.com/cgi-bin/PlantAligDB_taxonomicgroups.cgi). The users are able to quickly search and locate a queried feature, order each column using the ascendant or descendent mode, filter the information, download the curated datasets, among other features. The multiple sequence alignment and phylogenetic tree can be visualized by clicking in the number of species present in the alignment.

Genetic diversity

The PIS values in our current dataset vary from 0.16% to 99.07% (Table 2). Nevertheless, the PIS and PPI sequence conservation measures are not intended to be used for comparison of different families and/or regions, since the number of sequences in each alignment can be very different. The results must be interpreted at the light of the number of sequences and the representativeness of the sequences included in the alignment file. The *matK* was the region with the lowest PIS value (0.16%) [Figure 2. f)], while the *trnL* GH was the region with the highest PIS value (99.07%), as can be seen in Figure 2 d) and Table 2. The *rbcL* was the most conserved region [Figure 2 e)] with an average of 78.48%, while the ITS was less conserved with an average of 28.84% (Table 2). Our results are in accordance with earlier studies where *atpF-atpH* and *psbA-trnH* were found to be more variables than *matK* (Lahaye 2008). The *trnL* CD regions showed values slightly more conserved than *atpF-atpH* [Figure 2 c) and a)]. The lowest PPI value (69.96%) was found in *psbA-trnH* region [Figure 2 b)] and the highest was 100% in *trnL* GH, as shown in Figure 2 d) and Table 2. The ITS region was less conserved with an average of 87.21% [Table 3 and Figure 2 g)], while the *trnL* GH was the most conserved with an average of 97.09% [Table 3 d)].

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191 **Sequence alignments and phylogenetic trees**

192 The alignments stored in the database can be visualized using a dynamic browser-based
 193 application named Wasabi (<http://wasabiapp.org/>) (Veidenberg *et al.* 2016) with multiple
 194 options for the visualization and analysis of sequence data and phylogenetic trees. This
 195 resource is particularly useful to help researchers selecting the most appropriated genomic
 196 regions for their investigations. The users can zoom in and out the selected regions of the
 197 alignments, collapse regions with gaps, alternate between column and row selection, remove
 198 or add sequences, realign sequences, and export the sequence data in the FASTA format. If an
 199 Wasabi account is created, the user can re-align specific alignments with PAGAN (Loytynoja
 200 *et al.* 2012) and PRANK (Loytynoja 2014). The user can merge different alignments from
 201 identical regions (Hollingsworth *et al.* 2009) and different families using the PAGAN
 202 application. The download of the complete database of curated alignments is accessible in the
 203 *Download* section of the database.

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206 **How to use the PlantAligDB**

207 To explore a genomic region, a researcher must start by accessing the ‘Genomic
 208 Regions’ section in the menu bar. For example, by selecting *psbA-trnH*, the user will find a
 209 brief description of the genomic region and the name, size and position of that region in the
 210 reference genome, and the families with available alignments. The user should select the
 211 ‘Taxonomic Groups’ section in the menu bar to search for a specific plant family. Then,
 212 through the search tool on the right side of the page, the user can type the name of the family
 213 and a dynamic table will be displayed with the number of sequences for each region. The user
 214 can also access a hyperlink with the description of the family and taxonomic tree using the

resource Tree of Life Web Project (<http://tolweb.org>). Clicking on the number of sequences, the database is redirected to the Wasabi tool. The user can create a Wasabi account by providing an e-mail or choosing a temporary account, which allows to realign sequences with PAGAN or PRANK. The user can merge a PAGAN realignment with alignments of other families on the same region, by selecting a file on his local computer in the “alignment extension” option.

Availability and design

The PlantAligDB is freely available at <http://plantaligdb.portugene.com> and is optimized for the major web browsers (Internet Explorer, Firefox, Safari, and Chrome). The SQLite local database is used for data storage and runs on an Apache web server. The dynamic HTML pages were implemented using CGI-Perl and JavaScript and the dataset table views were generated using the JQuery plugin DataTables v1.9.4 (<http://datatables.net/>). The PlantAligDB visualization tables are generated automatically. The process of database update is optimized for large datasets. There are no access restrictions for academic and commercial use.

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Author Contributions

CS, JC and FP contributed equally to designed and performed research, analysed data and wrote the paper.

References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* **215**, 403-410.
- Domenech B, Asmussen-Lange CB, Baker WJ, *et al.* (2014) A phylogenetic analysis of palm subtribe Archontophoenicinae (Arecaceae) based on 14 DNA regions. *Botanical Journal of the Linnean Society* **175**, 469-481.
- Dong W, Liu J, Yu J, Wang L, Zhou S (2012) Highly variable chloroplast markers for evaluating plant phylogeny at low taxonomic levels and for DNA barcoding. *PLoS One* **7**, e35071.
- Drummond AB, Cheung M, Heled J, Kearse M, Moir R, Stones-Havas S, Thierer T, Wilson A (2009) Geneious Pro 4.8.2.
- Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* **32**, 1792-1797.
- Egan AN, Schlueter J, Spooner DM (2012) Applications of next-generation sequencing in plant biology. *Am J Bot* **99**, 175-185.
- Feuillet C, Leach JE, Rogers J, Schnable PS, Eversole K (2011) Crop genome sequencing: lessons and rationales. *Trends Plant Sci* **16**, 77-88.
- Group CPW (2009) A DNA barcode for land plants. *Proc Natl Acad Sci U S A* **106**, 12794-12797.
- Group CPW, Hollingsworth PM, Forrest LL, *et al.* (2009) A DNA barcode for land plants. *Proceedings of the National Academy of Sciences* **106**, 12794-12797.
- Hollingsworth ML, Andra Clark A, Forrest LL, *et al.* (2009) Selecting barcoding loci for plants: evaluation of seven candidate loci with species-level sampling in three divergent groups of land plants. *Mol Ecol Resour* **9**, 439-457.
- Hollingsworth PM, Graham SW, Little DP (2011) Choosing and using a plant DNA barcode. *PLoS One* **6**, e19254.
- Karehed J, Groeninckx I, Dessein S, Motley TJ, Bremer B (2008) The phylogenetic utility of chloroplast and nuclear DNA markers and the phylogeny of the Rubiaceae tribe Spermacoceae. *Mol Phylogenet Evol* **49**, 843-866.
- Lahaye RS, V.; Duthoit, S.; Maurin, O. and van der Bank, M. (2008) A test of psbK-psbI and atpF-atpH as potential plant DNA barcodes using the flora of the Kruger National Park as a model system (South Africa). *Nature Precedings*, 21.
- Lai K, Berkman PJ, Lorenc MT, *et al.* (2012) WheatGenome.info: an integrated database and portal for wheat genome information. *Plant Cell Physiol* **53**, e2.
- Li J, Gale SW, Kumar P, Zhang J, Fischer G (2018) Prioritizing the orchids of a biodiversity hotspot for conservation based on phylogenetic history and extinction risk. *Botanical Journal of the Linnean Society*, box084-box084.
- Loytynoja A (2014) Phylogeny-aware alignment with PRANK. *Methods Mol Biol* **1079**, 155-170.
- Loytynoja A, Vilella AJ, Goldman N (2012) Accurate extension of multiple sequence alignments using a phylogeny-aware graph algorithm. *Bioinformatics* **28**, 1684-1691.

284 Meyer S, Nagel A, Gebhardt C (2005) PoMaMo--a comprehensive database for potato genome data.
 285 *Nucleic Acids Res* **33**, D666-670.
 286 Numa H, Itoh T (2014) MEGANTE: a web-based system for integrated plant genome annotation.
 287 *Plant Cell Physiol* **55**, e2.
 288 Ratnasingham S, Hebert PD (2007) bold: The Barcode of Life Data System
 289 (<http://www.barcodinglife.org>). *Mol Ecol Notes* **7**, 355-364.
 290 Sakai H, Lee SS, Tanaka T, *et al.* (2013) Rice Annotation Project Database (RAP-DB): an integrative
 291 and interactive database for rice genomics. *Plant Cell Physiol* **54**, e6.
 292 Taberlet P, Coissac E, Pompanon F, *et al.* (2007) Power and limitations of the chloroplast trnL (UAA)
 293 intron for plant DNA barcoding. *Nucleic Acids Res* **35**, e14.
 294 Veidenberg A, Medlar A, Loytynoja A (2016) Wasabi: An Integrated Platform for Evolutionary
 295 Sequence Analysis and Data Visualization. *Mol Biol Evol* **33**, 1126-1130.
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Tables

Table 1: Summary of data currently available in the PlantAligDB.

Target region	Genome	Type	Length (bp) in <i>Nicotiana tabacum</i>	Number of alignments	Number of sequences
<i>atpF-atpH</i>	cpDNA	Inter-genic spacer	502	31	1025
<i>psbA-trnH</i>	cpDNA	Inter-genic spacer	509	79	4852
<i>trnL CD</i>	cpDNA	Intron	577	44	2527
<i>trnL GH</i>	cpDNA	Intron	78	173	34674
<i>rbcL</i>	cpDNA	Protein-coding gene	1434	39	1748
<i>matK</i>	cpDNA	Protein-coding gene	1530	113	11341
ITS	nuDNA	Transcribed spacers and 5.8S gene	678	35	9885
			<i>Total</i>	514	66052

Table 2: Average percentage of identical sites (PIS) values in all plant families organized by genomic region.

PIS	<i>atpF-atpH</i>	<i>psbA-trnH</i>	<i>trnL CD</i>	<i>trnL GH</i>	<i>rbcL</i>	<i>matK</i>	ITS
Mean	55.05	39.1	61.13	58.62	78.48	65	28.84
Max	85.95	97.42	96.46	99.07	95.24	97.3	78.45
Min	15.19	2.3	21	6.43	27.47	0.16	1.13

Table 3: Average percentage of pairwise identity (PPI) values in all plant families organized by genomic region.

PPI	<i>atpF-atpH</i>	<i>psbA-trnH</i>	<i>trnL CD</i>	<i>trnL GH</i>	<i>rbcL</i>	<i>matK</i>	ITS
Mean	95.49	94.02	96.34	97.09	96.94	95.23	87.21
Max	99.37	99.94	99.6	100	99.48	99.62	97.75
Min	87.59	69.96	90.54	88.54	81.18	78.72	70.79

Legends to figures

Figure 1: Workflow used to generate the curated alignments, phylogenetic trees, and genetic conservation values stored in PlantAligDB.

Figure 2: Graphic representation of the measures of sequence conservation PPI and PIS for each region-family alignment: a) *atpF-atpH* region b), *psbA-trnH* region, c) *trnL* CD region, d) *trnL* GH region, e) *rbcL* region, f) *matK* region and g) ITS region.

Selection of target genomic regions

DNA sequences obtained in public databases

Manual curation

removal of duplicated sequences from the same species
removal of sequences without a clear species assignment
reverse complement of sequences found in the opposite direction

Organization of sequences by family according to the NCBI taxonomy

Selection of families represented by ten or more species

Alignment of sequences in each family

Manual curation

removal of sequences that do not cover the entire target region
removal of sequences with large stretches of nucleotide ambiguities

Final sequence alignment

Figure 1

Figure 2