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 webservers, 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.

72 We describe on-line database (PlantAligDB, available an at 73 http://plantaligdb.portugene.com) with a comprehensive, automatically curated and regularly 74 updated collection of alignments from diverse plant families (Figure 1), and respective 75 phylogenetic inference of each alignment. The PlantAligDB is a consistent repository of 76 curated alignments and phylogenetic trees that are generated by the same workflow. For 77 example, the Orchidaceae family is frequently referred to as a critical group, whose species 78 are difficult to identify. Because of their ecological importance, ongoing studies continue to 79 be made to reduce their extinction risk and maintain their diversity (Li et al. 2018). The 80 PlantAligDB can help researchers designing accurate methods for plant identification (matK 81 and *rbcL* are used in DNA barcoding projects) whether by identification of conserved motives 82 that enable the design of primers. The PlantAligDB alignments can be used as a reference 83 database for phylogenetic studies, allowing the construction of reference phylogenetic trees of 84 the different regions [e.g., genomic regions atpF-atpH (Domenech et al. 2014), psbA-trnH 85 (Dong et al. 2012) and ITS (Karehed et al. 2008)]. Moreover, it provides useful data to 86 understand the genetic diversity of the selected genomic regions.

87

88 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 *trn*L (UAA) region (more 114 than 50,000 hits), we used the external regions named "C" and "D" and the internal regions

115 named "G" and "H" by (Taberlet et al. 2007) as queries in the NCBI Basic Local Alignment 116 Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/). The search was made against the 117 nucleotide collection (nr/nt) of Tracheophyta (vascular plants) using the Biopython package 118 (www.biopython.org) with an expected threshold of 1000 and a minimum word size of 16. 119 Therefore, our database includes two datasets for the *trn*L (UAA) genomic region: the '*trn*L 120 CD' target region with a length of 577 bp in N. tabacum, and the 'trnL GH' with a length of 121 78 bp in *N. tabacum*, located inside the *trn*L CD region. All information regarding the selected 122 target regions can be found in the *Genomic Regions* section of the PlantAligDB.

123 The nucleotide sequences of the six regions were organized by family according to the 124 NCBI taxonomy and were aligned (each region and family in separated alignments) using the 125 default parameters of the MUSCLE software (Edgar 2004) running in the Geneious software. 126 The alignment was repeated in some families after excluding sequences that do not cover the 127 entire region of interest and that had large stretches of nucleotide ambiguities. We only used 128 alignments with ten or more species per family to build the PlantAligDB. Some species 129 sequences were lost in this process filter. The neighbor joining phylogenetic tree of each 130 region-family were calculated using Tamura-Nei model using Geneious Tree Builder. The 131 methodology was built in Armadillo Workflow (http://www.bioinfo.uqam.ca/armadillo/) to 132 automate the update process of the database. The latest release update of June 2018 133 incorporates 514 alignments and phylogenetic trees, from 223 plant families.

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135 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

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

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- 142 **Results and Discussion**
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- 144 **Database organization**

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146 **Basic structure**

147 The PlantAligDB database is divided in nine sections (Figure 1): 1) Home, provides a 148 brief description of what can be done in the database; 2) Genomic regions, describes the 149 regions used in the database; 3) Taxonomic groups, the table containing the plant 150 family/region alignments and phylogenetic trees; 4) BLAST, search the database using a query 151 sequence by means of the BLASTN algorithm (Altschul et al. 1990); 5) Genetic diversity, 152 describes the percentage of identical sites (PIS) and pairwise identity values (PPI) for each 153 alignment; 6) Download, provides hyperlinks to download the curated alignments; 7) 154 Tutorials, contains information about how the database was built, and how to use it; 8) 155 Citations; 9) Contacts.

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157 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 165 the highest number of sequences. The alignments for each plant family can be accessed 166 through a dynamic table in the *Taxonomic groups* section of the database by following a 167 hyperlink with number of species included the in each alignment 168 (http://plantaligdb.portugene.com/cgi-bin/PlantAligDB taxonomicgroups.cgi). The users are 169 able to quickly search and locate a queried feature, order each column using the ascendant or 170 descendent mode, filter the information, download the curated datasets, among other features. 171 The multiple sequence alignment and phylogenetic tree can be visualized by clicking in the 172 number of species present in the alignment.

173

174 Genetic diversity

175 The PIS values in our current dataset vary from 0.16% to 99.07% (Table 2). 176 Nevertheless, the PIS and PPI sequence conservation measures are not intended to be used for 177 comparison of different families and/or regions, since the number of sequences in each 178 alignment can be very different. The results must be interpreted at the light of the number of 179 sequences and the representativeness of the sequences included in the alignment file. The 180 matK was the region with the lowest PIS value (0.16%) [Figure 2. f)], while the trnL GH was 181 the region with the highest PIS value (99.07%), as can be seen in Figure 2 d) and Table 2. The 182 rbcL was the most conserved region [Figure 2 e)] with an average of 78.48%, while the ITS 183 was less conserved with an average of 28.84% (Table 2). Our results are in accordance with 184 earlier studies where *atp*F-*atp*H and *psbA-trn*H were found to be more variables than *mat*K 185 (Lahaye 2008). The *trnL* CD regions showed values slightly more conserved than *atpF-atpH* 186 [Figure 2 c) and a)]. The lowest PPI value (69.96%) was found in *psbA-trn*H region [Figure 2 187 b)] and the highest was 100% in trnL GH, as shown in Figure 2 d) and Table 2. The ITS 188 region was less conserved with an average of 87.21% [Table 3 and Figure 2 g)], while the 189 *trn*L 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-trn*H, 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

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

221

222 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.

230

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242 Author Contributions

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

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298 299 Tables

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

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

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

PIS	atpF-atpH	psbA-trnH	trnL CD	trnL GH	<i>rbc</i> L	matK	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	atpF-atpH	psbA-trnH	trnL CD	trnL GH	<i>rbc</i> L	matK	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

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315 Legends to figures

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Figure 1: Workflow used to generate the curated alignments, phylogenetic trees, and genetic

318 conservation values stored in PlantAligDB.

- 320 Figure 2: Graphic representation of the measures of sequence conservation PPI and PIS for each
- 321 region-family alignment: a) *atpF-atpH* region b), *psbA-trnH* region, c) *trnL* CD region, d) *trnL* GH
- 322 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