High contiguity *de novo* genome sequence assembly of Trifoliate yam (*Dioscorea dumetorum*) using long read sequencing

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Abstract: The yam species *Dioscorea dumetorum* is one example of an orphan crop, not traded internationally. Post-harvest hardening starts within 24 hours after harvesting and renders the tubers inedible. Genomic resources are required for trifoliate yam to improve breeding for non-hardening varieties and for other traits. Here, we describe the sequencing of the *D. dumetorum* genome and the generation of a *de novo* assembly together with a corresponding annotation. The two haplophases of this highly heterozygous genome are separated to a large extent. The assembly represents 485 Mbp of the genome with an N50 of over 3.2 Mbp. A total of 35,269 protein-encoding gene structures as well as 9,941 non-coding RNA genes were predicted and functional annotations were assigned.

Keywords: yam; *D. dumetorum*; nanopore sequencing; genome assembly; comparative genomics; read depth

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1. Introduction

The yam species *Dioscorea dumetorum* (trifoliate yam) belongs to the genus *Dioscorea* comprising about 600 described species. The genus is widely distributed throughout the tropics [1] and includes important root crops that offer staple food for over 300 million people. Eight *Dioscorea* species are commonly consumed in West and Central Africa, of which *D. dumetorum* has the highest nutrient value [2]. Yet, *D. dumetorum* constitutes an underutilized and neglected species despite its great potential for nutritional, agricultural, and pharmaceutical purposes. Tubers of *D. dumetorum* are protein-rich (9.6%) with a fairly balanced essential amino acids composition [3]. The provitamin A and carotenoids contents of the tubers of deep yellow genotypes are equivalent to those of yellow corn maize lines selected for increased concentrations of provitamin A [4]. The deep yellow yam tubers are used in antidiabetic treatments in Nigeria [5], probably due to the presence of dioscoretine, which is a bioactive compound with hypoglycaemic properties [6].

Unlike other yam species, the cultivation of *D. dumetorum* is limited by post-harvest hardening, which starts within 24 h after harvest and renders tubers inedible. Previous research showed that among 32 *D. dumetorum* cultivars tested, one cultivar was not affected by the hardening phenomenon [7]. This discovery provides a starting point for a breeding program of *D. dumetorum* against the post-harvest hardening phenomenon. *Dioscorea* cultivars are obligate outcrossing plants that display highly heterozygous genomes. Thus, methods of genetic analysis routinely used in inbreeding species such as linkage analysis using the segregation progeny of an F2 generation and recombinant inbred lines are inapplicable to yam [8]. Furthermore, the development of marker-assisted selection requires the establishment of marker assays and dense genetic linkage maps. Thus, access to a complete and well-annotated genome sequence is one essential step towards the implementation of up-to-date genetic and genomic approaches for *D. dumetorum* breeding. Although the plastome sequences of 14 *Dioscorea* species [9] and the nuclear genome sequence of *Dioscorea rotundata* [8] have been published, there is still a need for data from additional, phylogenetically unrelated yam species. Here, we report sequencing and *de novo* assembly of the *D. dumetorum* Ibo sweet 3 genome sequence based on long reads.

2. Materials and Methods

2.1. Sampling and Sequencing

A diploid *D. dumetorum* accession Ibo sweet 3 that does not display post-harvest hardening was collected in the South-West region of Cameroon in 2013 [7]. Tubers of this accession were transferred to Oldenburg (Germany) and the corresponding plants were cultivated in a greenhouse at 25°C. The haploid genome size of the Ibo sweet 3 genotype had been estimated to be 322 Mbp through flow cytometry [10].

DNA was extracted from 1g of leaf tissue using a CTAB-based method modified from [11]. After grinding the sample in liquid nitrogen, the powder was suspended in 5mL CTAB1 buffer supplemented with 300µL ß-mercaptoethanol. The suspension was incubated at 75°C for 30 minutes and inverted every five minutes. Next, 5mL dichloromethane were added and the solutions were mixed by inverting. The sample was centrifuged at 11,200 g at 20°C for 30 minutes. The clear supernatant was mixed with 10mL CTAB2 in a new reaction tube by inverting. Next, a centrifugation was performed at 11,200 g at 20°C for 30 minutes. After discarding the supernatant, 1mL NaCl (1M) was added to re-suspend the sediment by gently flicking the tube. By adding an equivalent amount of 1mL isopropanol and careful mixing, the DNA was precipitated again and the sample was centrifuged as described above. After washing the sediment with 1mL of 70% ethanol, 200µL CTAB-TE buffer containing 2 mg RNaseA were added. Re-suspension and RNA degradation were achieved by incubation over night at room temperature. DNA quality and quantity were assessed via NanoDrop2000 measurement, agarose gel electrophoresis, and Qubit measurement. The SRE kit (Circulomics) was used to enrich long DNA fragments following the suppliers' instructions. Results were validated via Qubit measurement.

Library preparation was performed with 1µg of high molecular weight DNA following the SQK-LSK109 protocol (Oxford Nanopore Technologies, ONT). Sequencing was performed on four R9.4.1 flow cells on a GridION. Flow cells were treated with nuclease flush (20µL DNaseI (NEB) and 380µL nuclease flush buffer), once the number of active pores dropped below 200, to allow successive sequencing of multiple libraries on an individual flow cell. Live base calling was performed on the GridION by Guppy (ONT).

The Illumina paired-end (PE) library preparation was performed according to the Illumina TruSeq DNA Sample Preparation v2 Guide. High molecular weight DNA was fragmented by nebulization. End pair and A-tailing adaptors were ligated to the fragmented DNA. A two percent low melt agarose gel was used to size select adaptor-ligated fragments. The fragments harbouring adaptors on the both ends were enriched by PCR and final libraries were evaluated using PicoGreen. Average fragment size of the libraries was estimated on a Bio Analyzer High Sensitivity DNA chip. The PE library with an insert size of 700 to 790 bp was sequenced with 2 x 250 nt mode on an Illumina HiSeq-1500.

2.2. Genome assembly and polishing

Canu v1.8 [12] was deployed for the genome assembly with the following parameters "'genomeSize = 350m', 'corOutCoverage = 200' 'correctedErrorRate = 0.12' batOptions = -dg 3 -db 3 -dr 1 -ca 500 -cp 50' 'minReadLength = 10000' 'minOverlapLength = 5000' 'corMhapFilterThreshold = 0.0000000002' 'ovlMerThreshold = 500' 'corMhapOptions = --threshold 0.85 –num-hashes 512 –num-min-matches 3 –ordered-sketch-size 1000 –ordered-kmer-size 14 –min-olap-length 5000 –repeat-idf-scale 50'". The value for the genome size, estimated to be 322 Mbp, was increased to 350 Mbp to increase the number of reads utilized for the assembly process.

ONT reads were mapped back to the assembled sequence with minimap2 v2.17 [13], using the settings recommended for ONT reads. Next, the contigs were polished by racon v.1.4.7 [14] with -m 8 -x -6 -g -8 as recommended prior to the polishing step with medaka. Two runs of medaka v.0.10.0 (https://github.com/nanoporetech/medaka) polishing were performed with default parameters (-m r941_min_high) using ONT reads. Illumina short reads were aligned to the medaka consensus sequence using BWA-MEM v. 0.7.17 [15]. This alignment was subjected to Pilon v1.23 [16] for final polishing in three iterative rounds.

Downstream processing was based on a previously described workflow [17] and performed by customized Python scripts for purging of short contigs (<100kb) and calculation of assembly statistics (https://github.com/bpucker/yam). Contigs with less than 3-fold average coverage in an Illumina short read mapping were compared against nt via BLASTn with an e-value cut-off at 10^{-10} to identify and remove bacterial and fungal sequences.

2.3. Genome sequence annotation

Hints for gene prediction were generated by aligning *D. rotundata* transcript sequences (TDr96 v1.0 [8] as previously described [18]. BUSCO v3 [19] was applied to generate a species-specific parameter set. For comparison, the *D. rotundata* genome assembly GCA_002260605.1 [8] was retrieved from NCBI. Hints and parameters were subjected to AUGUSTUS v.3.3 [20] for gene prediction with previously described parameters [18]. Various approaches involving parameter files of rice and maize as well as running the gene prediction on a sequence with masked repeats were evaluated. BUSCO was applied again to assess the completeness of the gene prediction. The best results for *D. dumetorum* genome sequence annotation were obtained based on an unmasked assembly sequence with yam specific parameters generated via BUSCO as previously described [19,21]. Predicted genes were filtered based on sequence similarity to entries in several databases (UniProt/SwissProt, Araport11, *Brachypodium distachyon* v3.0, *Elaeis guineensis* v5.1, GCF_000005425.2, GCF_000413155.1, *Musa acuminata* Pahang v2). Predicted peptide sequences were compared to these databases via BLASTp [22] using an e-value cut-off of 10^{-5}. Scores of resulting BLASTp hits were normalized to the score when searched against the set of predicted peptides. Only predicted sequences with at least 0.25 score ratio and 0.25 query length covered by the best alignment were kept. Functional annotation was assigned via InterProScan5 [23] and through sequence similarity to well characterized sequences. Representative transcript and peptide sequences were identified per gene based on previously defined criteria to encode the longest possible peptide [24, Pucker, 2017 #5337].

Prediction of non-protein coding RNA genes like tRNA and rRNA genes was performed based on tRNAscan-SE v2.0.3 [25,26] and INFERNAL (cmscan) v1.1.2 [27] based on the Rfam13 [28].

2.4. Assembly and annotation assessment

The percentage of phased and merged regions in the genome was assessed with the focus on predicted genes. Based on Illumina and ONT read mappings, the average coverage depth per gene was calculated. The distribution of these average values per gene allowed the classification of genes as phased (haploid read depth) or merged (diploid read depth). As previous studies revealed that Illumina short reads have a higher resolution for such coverage analysis [29], we focused on the Illumina read data set for these analyses. Sequence variants were detected based on this read mapping as previously described [30]. The number of heterozygous variants per gene was calculated and compared between the groups of putatively phased and merged genes. Predicted peptide sequences were compared against the annotation of other species including *A. thaliana* and *D. rotundata* via OrthoFinder v2 [31].

Sequence reads and assembled sequences are available at ENA under the project ID ERP118030 (see File S1 for details). The assembly described in this manuscript is available under GCA_902712375. Additional annotation files are available from https://docs.cebitec.uni-bielefeld.de/s/ArHmB4J2MXMsA5S.

Alleles covered by the fraction of phase separated gene structures were matched based on reciprocal best BLAST hits of the coding sequences following a previously described approach [17]. Alleles were considered a valid pair that represents a single gene if the second best match displayed 99% or less of the score of the best match. A customized Python script for this allele assignment is available on github (https://github.com/bpucker/yam).

3. Results

In total, we generated 70 Gbp of ONT reads data representing respectively about 218x coverage of the estimated 322 haploid genome. Additionally, 13 Gbp of Illumina short read data (about 40x coverage) were generated. After all polishing steps, the final assembly represents 485 Mbp of the highly heterozygous *D. dumetorum* genome with an N50 of 3.2 Mbp (Table 1). Substantial improvement of the initial assembly through various polishing steps is indicated by the increasing number of recovered BUSCOs (File S2). The final assembly displayed more BUSCOs (92.30% out of 1440 included in the embryophyta data set) compared to the publicly available genome sequence assembly of *D. rotundata* (v0.1) for that we detected 81.70% BUSCOs with identical parameters.

Table 1. Statistics of selected versions of the *D. dumetorum* genome assembly (see File S3 for a full table).

	Initial assembly	Racon1	Medaka2	Pilon3	Final
Number of contigs	1,172	1,172	1,215	1,215	924
Max. contig length [bp]	20,187,448	20,424,333	17,910,017	17,878,854	17,878,854
Assembly size [bp]	501,985,705	508,061,170	507,215,754	506,184,192	485,115,345
Assembly size without N [bp]	501,985,705	508,061,170	507,215,754	506,184,192	485,115,345
GC content	37.74%	37.66%	37.87%	37.59%	37.57%
N50 [bp]	3,896,882	3,930,287	2,598,889	2,593,751	3,190,870
N90 [bp]	136,614	138,199	137,206	136,754	156,407
BUSCO (complete)	85.70%	89.80%	91.90%	92.30%	92.30%

Different gene prediction approaches were evaluated (File S4) leading to a final set of 35,269 protein-encoding gene structures. The average gene spans 4.3 kbp, comprises 6 exons and encodes 455 amino acids (see File S4 for details). The gene prediction dataset for *D. dumetorum* is further supported by the identification of 6,475 single copy orthologs between *D. dumetorum* and *D. rotundata* as well as additional orthogroups (File S5). If the phase separated allelic gene structures were considered (Figure 1), 3,352 additional single copy orthologs were detected. Functional annotation was assigned to 23,835 genes (File S6). Additionally, 9,941 non-coding RNA genes were predicted including 784 putative tRNA genes (https://docs.cebitec.uni-bielefeld.de/s/ArHmB4J2MXMsA5S).

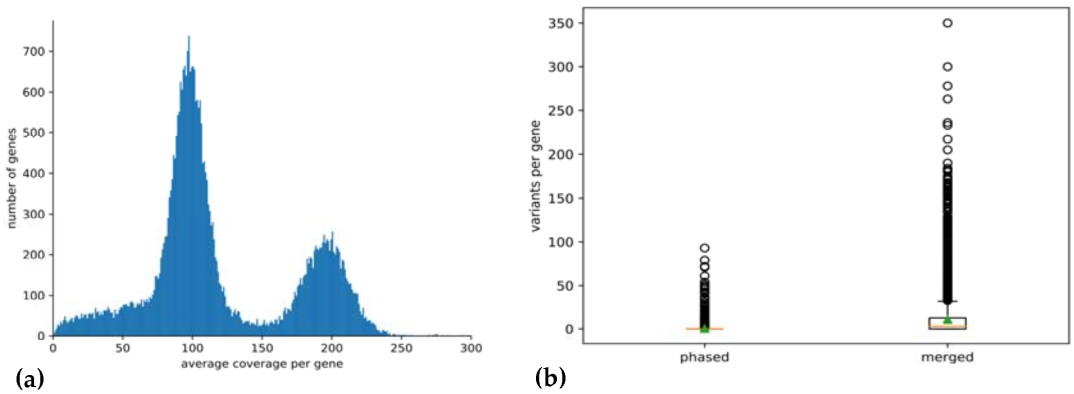

Figure 1. (**a**) Distribution of the average sequencing read depth per gene structure. Predicted gene structures were classified into phase separated and merged based on the average read depth value deduced from the analysis presented here. The haploid read depth with Illumina short reads ranges from 50-fold to 150-fold. (**b**) Number of heterozygous sequence variants in phase separated and merged genes. The high proportion of heterozygous variants in merged genes is due to the mapping of reads originating from two different alleles to the same region of the assembly.

Average read mapping depth per gene was analyzed to distinguish genes annotated in separated and merged haplophases, respectively (Figure 1, File S7). About 64% of all predicted protein-encoding gene structures were in the expected range of the haploid read mapping depth between 50-fold and 150-fold and about 27% are merged with a read depth between 150-fold and 250-fold. Only 6% of all genes show an average read depth below 50-fold and only 1% show an average coverage higher than 250-fold. It should be noted that the gene structures annotated in the phase separated part will cover in general two alleles per gene. A total of 22,885 gene structures, representing the 64% in the range of the haploid read mapping depth, were sorted into allelic pairs which was successful for 8,492 genes.

4. Discussion

The release of genome sequences of many model and crop plants has provided new opportunities for gene identification and studies of genome evolution, both ultimately serving the process of plant breeding [32] by allowing discovery of genes responsible for important agronomic traits and the development of molecular markers associated with these traits. Here, we present the first genome sequence for *Dioscorea dumetorum*, an important crop for Central and Western Africa, and the second for the genus. Our assembly offers a great opportunity to understand the evolution of yam and to elucidate some biological constraints inherent to yam including a long growth cycle, poor to non-flowering, polyploidy, vegetative propagation, and a heterozygous genetic background [33]. Yam improvement has been challenging due to these factors preventing the genetic study of important traits in yam [34].

Oxford Nanopore sequencing has proven to be a reliable and affordable technology for sequencing genomes thus replacing Illumina technique for *de novo* genome sequencing due to substantially higher assembly continuity [29,35]. Large fractions of the genome sequence were separated into phases, while regions with lower heterozygosity are merged into one representative sequence. Coverage analysis with Illumina read mapping allowed to classify predicted gene structures as 'phased' or 'merged' based on an average coverage around 100 fold or around 200 fold, respectively. While this distinction is possible at the gene structure level, whole contigs cannot be classified this way. Several million bp long contigs comprise alternating phase separated and merged regions. Therefore, it is likely that the contigs represent a mixture of both haplophases with the risk of switching between phases at each merged region. Since the haplophases cannot be resolved continuously through low heterozygosity regions, purging of contigs to reduce the assembly into a representation of the haploid genome might be advantageous for some applications in the future. The bimodal coverage distribution (Figure 1a) supports the assumption that *D. dumetorum* Ibo sweet 3 has a diploid genome. A higher ploidy would result in more distinct coverage peaks as observed for a genome with up to pentaploid parts [29]. The N50 of 3.2 Mbp is in the expected range for a long read assembly of a highly heterozygous species as others reported similar values before [36]. Due to regions of merged haplophases the total assembly size of 485 Mbp is smaller than expected for a fully phase separated "diploid" genome sequence based on the haploid genome size estimation of 322 Mbp.

Interestingly, we noticed an increase of the number of BUSCOs through several polishing rounds. Initial assemblies of long reads can contain numerous short insertions and deletions as these are the major error type [37]. The identification of open reading frames is hindered through apparent disruptions of open reading frames. Through the applied polishing steps, the number of such apparent frame shifts is reduced thus leading to an increase of detected BUSCOs.

Dioscorea dumetorum has 36 chromosomes [38], so with 924 contigs we are far from chromosome-level resolution but considerably better than the other genome assembly published in the genus, that of *D. rotundata* with 40 chromosomes [8]. Knuth [39], circumscribed *D. dumetorum* and *D. rotundata* in two sections *Lasiophyton* and *Enantiophlyllum* respectively. Also, phylogenetically the two species are quite distantly related [9]. Comparing our predicted peptides to the *D. rotundata* peptide set [8], we identified about 9,800 single copy orthologs (6,475 in the whole set of 35,269 gene structures plus 3,352 with a relation of one gene in *D. rotundata* and two phase separated alleles in *D. dumetorum*) which could elucidate the evolutionary history of those species. Our genome sequence is structurally accurate and more protein-encoding genes were predicted. The number of predicted protein-encoding gene structures was determined to be 35,269, but this number includes two times about 11,300 genes (see Figure 1) represented by two alleles. The CDS-based pairing we performed detected about 8,500 of the theoretical maximum of 11.300 cases which is a good success rate given the fact that close paralogs and also hemizygous genome regions contribute to the detected number of phase separated gene structures. If phase separated gene structures (alleles) are excluded, a number of about 24,000 genes would result for *D. dumetorum*. This fits to the range detected in other higher plant genomes [40, Pucker, 2019 #5484]. The BUSCO results support this interpretation with about 40% of BUSCOs that occur with exactly two copies. Therefore, the true number of protein-encoding genes of a haploid yam genome could be around 25.000, also considering that the BUSCO analysis indicated that still a small fraction of the genome sequence is missing. This gene number fits well to gene numbers of higher plants based on all available annotations at the NCBI [41] and is larger than that of *Theobroma cacao*, *Jatropha curcas*, *Oryza brchyantha*, and *Ananas comosus*. The average length of genes and the number of encoded amino acids are in the same range as previously observed for other plant species from diverse taxonomic groups [21,42].

Our draft genome has the potential to provide a complete new way to breed in *D. dumetorum*, for example avoiding the post-harvest hardening phenomenon, which begins within 24 h after harvest and makes it necessary to process the tubers within this time to allow consumption [2]. The family Dioscoreaceae consists of more than 800 species [43] and the post-harvest hardening phenomenon has only been reported from *D. dumetorum* [44], outlining the singularity of this species among yam species. We predicted a large number of genes, which will include putative genes controlling the post-harvest hardening on *D. dumetorum* and many useful bioactive compounds detected in this yam species, which is considered the most nutritious and valuable from a phytomedical point of view [45]. Ongoing work will try to identify these genes and polymorphisms for making them available for subsequent breeding.

In summary, we present the first *de novo* nuclear genome sequence assembly of *D. dumetorum* with very good contiguity and partially separated phases. Our assembly has no ambiguous bases with a well applicable protein-encoding gene annotation. This assembly unraveled the genomic structure of *D. dumetorum* to a large extent and will serve as a reference genome sequence for yam breeding by helping to identify and develop molecular markers associated with relevant agronomic traits, and to understand the evolutionary history of *D. dumetorum* and yam species in general.

Supplementary Materials: The following are available online:
File S1: Sequencing overview with ENA identifiers of runs.
File S2: Results of BUSCO analysis of different assembly versions.
File S3: General statistics of different assembly versions.
File S4: Comparison of different gene prediction approaches.
File S5: Orthogroups of predicted peptides of D. rotundata and D. dumetorum.
File S6: Functional annotation of predicted genes in the D. dumetorum genome sequence.
File S7: Average short read mapping coverage of predicted genes in the D. dumetorum genome sequence.

Author Contributions: CS, BP, DCA, and BW designed the study. CS collected the sample. BP performed DNA extraction, ONT sequencing, and genome assembly. PV performed Illumina sequencing. CS and BP processed the assembly. BP performed gene prediction and evaluation. CS and BP wrote the initial draft. BW and DCA revised the manuscript. All authors read and approved the final version of the manuscript.

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References

1. Viruel, J.; Forest, F.; Paun, O.; Chase, M.W.; Devey, D.; Couto, R.S.; Segarra-Moragues, J.G.; Catalan, P.; Wilkin, P. A nuclear Xdh phylogenetic analysis of yams (Dioscorea Dioscoreaceae) congruent with plastid trees reveals a new Neotropical lineage. *Botanical Journal of the Linnean Society* **2018**, 1-15.
2. Sefa-Dedeh, S.; E.O., A. Biochemical and textural changes in trifoliate yam Dioscorea dumetorum tubers after harvest. *Food Chemistry* **2002**, *79*, 27-40.
3. Alozie, Y.E.; Akpanabiatu, M.; Eyong, E.U.; Umoh, I.B.; Alozie, G. Amino Acid Composition of Dioscorea dumetorum Varities. *Pakistan Journal of Nutrition* **2009**, *8*, 103-105.
4. Ferede, R.; Maziya-Dixon, B.; Alamu, O.E.; Asiedu, R. Identification and quantification of major carotenoids of deep yellow-fleshed yam (tropical Dioscorea dumetorum). *Journal of Food, Agruculture & Environment* **2010**, *8*, 160-166.

5. Nimenibo-Uadia, R.; Oriakhi, A. Proximate, Mineral and Phytochemical Composition of Dioscorea dumetorium Pax. *J. Appl. Sci. Environ. Manage.* **2017**, *21*, 771-774.
6. Iwu, M.M.; Okunji, C.O.; Ohiaeri, G.O.; Akah, P.; Corley, D.; Tempesta, M.S. Hypoglycaemic activity of dioscoretine from tubers of Dioscorea dumetorum in normal and alloxan diabetic rabbits. *Planta Medica* **1990**, *56*, 264-267.
7. Siadjeu, C.; Panyoo, E.A.; Toukam, G.M.S.; Bell, J.M.; Nono, B.; Medoua, G.N. Influence of Cultivar on the Postharvest Hardening of Trifoliate Yam (Dioscorea dumetorum) Tubers. *Hindawi* **2016**, *16*.
8. Tamiru, M.; Natsume, S.; Takagi, H.; White, B.; Yaegashi, H.; Shimizu, M.; Yoshida, K.; Uemura, A.; Oikawa, K.; Abe, A., et al. Genome sequencing of the staple food crop white Guinea yam enables the development of a molecular marker for sex determination. *BMC Biology* **2017**, *15*, 86.
9. Magwe-Tindo, J.; Wieringa, J.J.; Sonke, B.; Zapfack, L.; Vigouroux, Y.; Couvreur, T.L.P.; Scarcelli, N. Complete plastome sequence of 14 African yam species (Dioscorea spp.). *Mitochondrial DNA Part B* **2019**.
10. Siadjeu, C.; Mayland-Quellhorst, E.; Albach, D.C. Genetic diversity and population structure of trifoliate yam (Dioscorea dumetorum Kunth) in Cameroon revealed by genotyping-by-sequencing (GBS). *BMC Plant Biology* **2018**, *18*, 359.
11. Rosso, M.G.; Li, Y.; Strizhov, N.; Reiss, B.; Dekker, K.; Weisshaar, B. An *Arabidopsis thaliana* T-DNA mutagenised population (GABI-Kat) for flanking sequence tag based reverse genetics. *Plant Molecular Biology* **2003**, *53*, 247-259.
12. Koren, S.; Walenz, B.P.; Berlin, K.; Miller, J.R.; Bergman, N.H.; Phillippy, A.M. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. *Genome Research* **2017**, *27*, 722-736.
13. Li, H. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics* **2018**, *34*, 3094-3100.
14. Vaser, R.; Sović, I.; Nagarajan, N.; Šikić, M. Fast and accurate de novo genome assembly from long uncorrected reads. *Genome Research* **2017**, *27*, 737-746.
15. Li, H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv* **2013**, 1303.3997v1302 (Preprint posted May 1326, 2013).
16. Walker, B.J.; Abeel, T.; Shea, T.; Priest, M.; Abouelliel, A.; Sakthikumar, S.; Cuomo, C.A.; Zeng, Q.; Wortman, J.; Young, S.K., et al. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS ONE* **2014**, *9*, e112963.
17. Pucker, B.; Holtgräwe, D.; Rosleff Sörensen, T.; Stracke, R.; Viehöver, P.; Weisshaar, B. A De Novo Genome Sequence Assembly of the Arabidopsis thaliana Accession Niederzenz-1 Displays Presence/Absence Variation and Strong Synteny. *PLoS ONE* **2016**, *11*, e0164321.
18. Pucker, B.; Holtgräwe, D.; Weisshaar, B. Consideration of non-canonical splice sites improves gene prediction on the Arabidopsis thaliana Niederzenz-1 genome sequence. *BMC Research Notes* **2017**, *10*, 667.
19. Simão, F.A.; Waterhouse, R.M.; Ioannidis, P.; Kriventseva, E.V.; Zdobnov, E.M. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* **2015**, *31*, 3210-3212.

20. Keller, O.; Kollmar, M.; Stanke, M.; Waack, S. A novel hybrid gene prediction method employing protein multiple sequence alignments. *Bioinformatics* **2011**, *27*, 757-763.
21. Pucker, B.; Feng, T.; Brockhington, S. Next generation sequencing to investigate genomic diversity in Caryophyllales. *bioRxiv* **2019**, , doi:10.1101/646133 (Preprint posted 2019-07-27).
22. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. *Journal of Molecular Biology* **1990**, *215*, 403-410.
23. Jones, P.; Binns, D.; Chang, H.Y.; Fraser, M.; Li, W.; McAnulla, C.; McWilliam, H.; Maslen, J.; Mitchell, A.; Nuka, G., et al. InterProScan 5: genome-scale protein function classification. *Bioinformatics* **2014**, *30*, 1236-1240, doi:10.1093/bioinformatics/btu031.
24. Cheng, C.Y.; Krishnakumar, V.; Chan, A.; Thibaud-Nissen, F.; Schobel, S.; Town, C.D. Araport11: a complete reannotation of the Arabidopsis thaliana reference genome. *The Plant Journal* **2017**, 789-804.
25. Lowe, T.M.; Eddy, S.R. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Research* **1997**, *25*, 955-964.
26. Chan, P.P.; Lowe, T.M. tRNAscan-SE: Searching for tRNA genes in genomic sequences. In *Gene Prediction: Methods and Protocols*, 2019/04/26 ed.; Kollmar, M., Ed. Springer New York: New York, 2019; Vol. 1962, pp. 1-14.
27. Nawrocki, E.P.; Eddy, S.R. Infernal 1.1: 100-fold faster RNA homology searches. *Bioinformatics* **2013**, *29*, 2933-2935.
28. Kalvari, I.; Argasinska, J.; Quinones-Olvera, N.; Nawrocki, E.P.; Rivas, E.; Eddy, S.R.; Bateman, A.; Finn, R.D.; Petrov, A.I. Rfam 13.0: shifting to a genome-centric resource for non-coding RNA families. *Nucleic Acids Research* **2018**, *46*, D335-D342.
29. Pucker, B.; Ruckert, C.; Stracke, R.; Viehover, P.; Kalinowski, J.; Weisshaar, B. Twenty-Five Years of Propagation in Suspension Cell Culture Results in Substantial Alterations of the Arabidopsis Thaliana Genome. *Genes* **2019**, *10*, 671, doi:10.3390/genes10090671.
30. Baasner, J.S.; Howard, D.; Pucker, B. Influence of neighboring small sequence variants on functional impact prediction. *bioRxiv* **2019**, , doi:10.1101/596718 (Preprint posted 2019-06-13).
31. Emms, D.M.; Kelly, S. OrthoFinder: phylogenetic orthology inference for comparative genomics. *Genome Biology* **2019**, *20*, 238, doi:10.1186/s13059-019-1832-y.
32. Ruggieri, V.; Alexiou, K.G.; Morata, J.; Argyris, J.; Pujol, M.; Yano, R.; Nonaka, S.; Ezura, H.; Latrasse, D.; Boualem, A., et al. An improved assembly and annotation of the melon (Cucumis melo L.) reference genome. *Scientific Reports* **2018**, *8*, 8088, doi:10.1038/s41598-018-26416-2.
33. Mignouna, H.D.; Abang, M.M.; Asiedu, R. Harnessing modern biotechnology for tropical tuber crop improvement: Yam (Dioscorea spp.) molecular breeding. *African Journal of Biotechnology* **2003**, *2*, 12.
34. Mignouna, H.D.; Abang, M.M.; Asiedu, R. Genomics of Yams, a Common Source of Food and Medicine in the Tropics. In *Genomics of Tropical Crop Plants*, Moore, P.H., Ming, R., Eds. 2008; 10.1007/978-0-387-71219-2_23pp. 549-570.
35. Michael, T.P.; Jupe, F.; Bemm, F.; Motley, S.T.; Sandoval, J.P.; Lanz, C.; Loudet, O.; Weigel, D.; Ecker, J.R. High contiguity Arabidopsis thaliana genome assembly with a single nanopore flow cell. *Nature Communications* **2018**, *9*, 541.

36. Paajanen, P.; Kettleborough, G.; Lopez-Girona, E.; Giolai, M.; Heavens, D.; Baker, D.; Lister, A.; Cugliandolo, F.; Wilde, G.; Hein, I., et al. A critical comparison of technologies for a plant genome sequencing project. *Gigascience* **2019**, *8*, doi:10.1093/gigascience/giy163.
37. Salmela, L.; Walve, R.; Rivals, E.; Ukkonen, E. Accurate self-correction of errors in long reads using de Bruijn graphs. *Bioinformatics* **2017**, *33*, 799-806, doi:10.1093/bioinformatics/btw321.
38. Miege, J. Nombres chromosomiques et répartition géographique de quelques plantes tropicales et équatoriales. *Revue de Cytologie et de Biologie Végétales* **1954**, *15*, 312-348.
39. Knuth, R. Dioscoreaceae. In *Das Pflanzenreich*, Engelr, A., Ed. Engelmann, W.: Leipzig, 1924.
40. Wendel, J.F.; Jackson, S.A.; Meyers, B.C.; Wing, R.A. Evolution of plant genome architecture. *Genome Biology* **2016**, *17*, 37, doi:10.1186/s13059-016-0908-1.
41. Pucker, B.; Brockington, S.F. Genome-wide analyses supported by RNA-Seq reveal non-canonical splice sites in plant genomes. *BMC Genomics* **2018**, *19*, 980.
42. Pucker, B.; Holtgräwe, D.; Stadermann, K.B.; Frey, K.; Huettel, B.; Reinhardt, R.; Weisshaar, B. A chromosome-level sequence assembly reveals the structure of the Arabidopsis thaliana Nd-1 genome and its gene set. *PLoS One* **2019**, *14*, e0216233.
43. Barton, H. Yams: Origins and Development. **2014**.
44. Treche, S.; Delpeuch, F. Physiologie Vegetale - Mise en evidence de l'apparition d'un epaississement membranaire dans le parenchyme des tubercules de Dioscorea dumetorum au cours de la conservation. *C. R. Acad. Sc. Paris* **1979**, *288*.
45. Price, E.J.; Wilkin, P.; Sarasan, V.; Fraser, P.D. Metabolite profiling of Dioscorea (yam) species reveals underutilised biodiversity and renewable sources for high-value compounds. *Scientic Reports* **2016**, *6*.