A large set of 26 new reference transcriptomes dedicated to comparative population genomics in crops and wild relatives

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A large set of 26 new reference transcriptomes dedicated to comparative population genomics in crops and wild relatives

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A large set of 26 new reference transcriptomes dedicated to comparative population genomics in crops and wild relatives

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Running title. 26 new reference transcriptomes of plants

Abstract

We produced a unique large dataset of reference transcriptomes to obtain new knowledge about the evolution of plant genomes and crop domestication. For this purpose we validated a RNA-Seq data assembly protocol to perform comparative population genomics. For the validation, we assessed and compared the quality of *de novo* Illumina short-read assemblies using data from two crops for which an annotated reference genome was available, namely grapevine and sorghum. We used the same protocol for the release of 26 new transcriptomes of crop plants and wild relatives, including still understudied crops such as yam, pearl millet and fonio. The species list has a wide taxonomic representation with the inclusion of 15 monocots and 11 eudicots. All contigs were annotated using BLAST, prot4EST, and Blast2GO. A strong originality of the dataset is that each crop is associated with close relative species, which will permit whole genome comparative evolutionary studies between crops and their wild related species. This large resource will thus serve research communities working on both crops and model organisms. All the data are available at http://arcad-bioinformatics.southgreen.fr/.

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Introduction

Population genomic approaches including both crops and wild relatives are essential to provide key information on the crop domestication processes and the dynamics of recent adaptation (Ross-Ibarra *et al.* 2007). This has been applied to an increasing number of species but mainly annual crops, and especially cereals (Hufford *et al.* 2012). However, domestication scenarios may vary between species and more insights could be gained by comparative approaches in diverse taxa (Glemin & Bataillon 2009; Meyer *et al.* 2012). It could be also very fruitful to compare molecular evolutionary patterns among species with contrasted life-history or ecological traits. Life-history or ecological traits may influence genome evolution through their effect on key population genetic parameters (effective size, recombination rates, and mutation rates). Furthermore, studying the evolution of gene families and relating it to expression data across lineages may help to identify which molecular functions play a key role in adaptation. A crucial initial step for such studies is to obtain a large set of reference genes in different crops, both for wild and cultivated populations, but also in close relative species as much insight can be gained by comparing genomes beyond the domestication scale; for instance several selection detection methods require outgroup species (Vitti *et al.* 2013). Today, the massive sequencing technologies can produce essential resources for large comparative genomic studies among many species, including non-model species (Ellegren 2014).

We have made available a unique dataset of 26 new plant reference transcriptomes that were produced, assembled and annotated with the same protocol. We chose a set of various Mediterranean and tropical crop species with contrasted life-history traits (ex: annual herbs/trees, outcrossers/selfers) and with a wide phylogenetic distribution (including both monocots and eudicots belonging to different orders). For non-model

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crops (ex: yam, fonio millet), we chose both the crop and close relatives. For some species already having a reference genome (ex: tomato, cocoa) we focused on relative outgroup species (see detailed list in Table 1). We chose high-throughput RNA-Seq technology for feasibility reasons, for possibilities of obtaining the coding part of the genome and expression levels, and for comparing the fate of some genes in different species. As we were focusing on providing tools for comparative and population genomics analyses, we were particularly attentive to the optimization of the protocol for assembling the Illumina short-reads in order to reconstruct the best ratio of unambiguous correct cDNAs, which we favoured instead of the exhaustive representation of transcriptomes.

We assessed and compared the quality of *de novo* assemblies using data from two crops for which an annotated reference genome is available, grapevine and sorghum. This study allowed us to provide a useful set of plant transcriptomes, contributing both to the enrichment of genomic resources for various crops of agronomic interest (some of them having being neglected so far such as yam, fonio millet or einkorn wheat) and phylogenetically related species that can be used as outgroups, which are invaluable tools for many evolutionary studies (Table 1). Among the chosen crops, some of them have been neglected so far while they can contribute to taking up some current agronomic challenges. For example, the diploid einkorn wheat could provide a useful resource for polyploidy wheat breeding programs, underutilized African cereals such as fonio millet deserve to be developed as an alternative crop well adapted to tropical conditions, and yam is a good model to understand clonally propagated crops.

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Materials and Methods

Preparation of RNA samples

Samples were constituted by combining different organs, including leaves, fruits/grains, and inflorescence tissues. Samples were ground in liquid nitrogen and total cellular RNA was extracted using a Spectrum Plant Total RNA kit (Sigma, Inc., USA) with a DNAse treatment. Oil palm total RNAs were extracted in a similar way using an RNeasy kit (Qiagen) and *Mauritia flexuosa* total RNAs were extracted as described by Morcillo et al. (Morcillo *et al.* 2006). RNA concentration was first measured using a NanoDrop ND-1000 Spectrophotometer then with the Quant-iT™ RiboGreen® (invitrogen) protocol on a Tecan Genius spectrofluorimeter. RNA quality was assessed by running 1 µL of each RNA sample on RNA 6000 Pico chip on a Bioanalyzer 2100 (Agilent Technologies, Inc., USA). Samples with an RNA Integrity Number (RIN) value greater than eight were deemed acceptable according to the Illumina TruRNA-Seq protocol. Usually, for each genotype, a 2 µg mixture consisting of RNA from the inflorescence (80%) and leaves (20%) was prepared. For a few species, three sources of RNA were used: 65% from the inflorescence, 15 % from leaves, and 20% from fruits or grains when these additional organs were of specific agronomic interest and could increase the transcriptome representation.

Illumina library production

The TruSeq RNA sample Preparation v2 kit (Illumina Inc., USA) was used according to the manufacturer's protocol with the following modifications. Poly-A containing mRNA molecules were purified from 2 ug of total RNA using poly-T oligo attached magnetic beads. The purified mRNA was fragmented by addition of the fragmentation buffer and was heated at 94°C in a thermocycler for 4 min. A fragmentation time of 4 min was

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used to yield library fragments of 250-300 bp. First strand cDNA was synthesized using random primers to eliminate the general bias towards the 3' end of the transcript. Second strand cDNA synthesis, end repair, A-tailing, and adapter ligation was performed in accordance with the protocols supplied by the manufacturer. Purified cDNA templates were enriched by 15 cycles of PCR for 10 s at 98°C, 30 s at 65°C, and 30 s at 72°C using PE1.0 and PE2.0 primers, and with Phusion DNA polymerase. Each indexed cDNA library was verified and quantified using a DNA 100 Chip on a Bioanalyzer 2100 then equally mixed by ten (from different genotypes). The final library was then quantified by real time PCR with the KAPA Library Quantification Kit for Illumina Sequencing Platforms (Kapa Biosystems Ltd, SA) and adjusted to 10 nM in water prior to sequencing at the Montpellier Genomix platform (http://www.mgx.cnrs.fr/).

Illumina library clustering and sequencing conditions

The final mixed cDNA library was sequenced using the Illumina mRNA-Seq, paired-end protocol on a HiSeq2000 sequencer, for 2 x 100 cycles. The library was diluted to 2 nM with NaOH. 2.5 µL was transferred into 497.5 µL HT1 to give a final concentration of 10 pM. 120 µL was then transferred to a 200 µL strip tube and placed on ice before loading onto the Cluster Station. A mixed library consisting of 10 individually indexed libraries was run on a single lane. Flow cells were clustered using Paired-End Cluster Generation Kit V4, following the Illumina PE amplification Linearization Blocking PrimerHyb v7 recipe. Then, the flow cell was loaded onto the Illumina HiSeq 2000 instrument following the manufacturer's instructions. The sequencing chemistry used was v4 (FC-104-4001, Illumina) using SCS 2.6 and RTA 1.6 software with the 2 x 100 cycles, paired-end, indexed protocol. Illumina base calling files were processed using

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the GERALD pipeline to produce paired sequence files containing reads for each sample in Illumina FASTQ format.

Read cleaning

Reads were preprocessed with cutadapt (Martin 2011) using the TruSeq index sequence corresponding to the sample, searching within the whole sequence. We also used cutadapt to trim the end of the reads with low quality scores (parameter -q 20) and kept reads with a minimum length of 35 bp. We then filtered the reads on the basis of their mean quality score, keeping those with a mean quality higher than 30. Subsequently we separated orphan reads (i.e those for which the mate was discarded in the previous steps) using a homemade script.

Assembly protocols

Reads were assembled using Abyss (Simpson et al. 2009) followed by one step of Cap3 (Huang & Madan 1999). Reads returned as singletons by the first assembly run were discarded. For assembly, only paired reads were used. Abyss was launched using the paired-end option with a kmer value of 60. Cap3 was launched with the default parameters, notably 40 bases of overlap and the percentage of identity was set at 90%.

Comparison of assembly protocols

We compared different combinations using the assemblers Abyss (Simpson *et al.* 2009), Cap3 (Huang & Madan 1999), and Trinity (Haas *et al.* 2013), with different parameters. We compared assemblies with Trinity (T), Abyss (A), and Abyss followed by one step of Cap3 (A/C), and Abyss followed by two steps of Cap3 (A/C/C) according to the strategy of Cahais *et al.* (2012). We used these protocols with reads from either only one individual or from multiple individuals. When the assemblies with only one

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individual were used, we chose the individual with the highest read number after cleaning: EC4 for *Sorghum bicolor* (SSM1123) and VC2 for *Vitis vinifera* (Morenoa). When assemblies were performed with reads from multiple individuals, we used 10 individuals for *Sorghum bicolor* and 12 individuals for *Vitis vinifera* (Table 2). In both cases, all reads came from a single sequencing lane. Since sampling, bank preparation and sequencing conditions were the same for all individuals, we assumed that the individual with the highest read number would enable reconstruction of the maximum number of weakly expressed transcripts. To perform Trinity with reads from multiple individuals, we used in silico read normalization to reduce memory requirements and improve computational times. Trinity was launched with the default parameters, except that the threshold for minimum contig length was increased from 200 bp to 250 bp.

Criteria for comparing the quality of assemblies

For each assembly we calculated the number of contigs, the mean length of the contigs, the N50, and the number of contigs that mapped to the reference genome (see Exonerate mapping description below). We used BWA (Li & Durbin 2009) (3 mismatches allowed) to map the reads from 10 individuals for *Sorghum bicolor*, and 12 individuals for *Vitis vinifera* to the corresponding assessed contigs. For each assembly, we calculated the percentage of mapped reads and the percentage of correctly paired mapped reads. Correctly paired mapped reads were defined as being within 1000 bases of each other. We also mapped the reads to the transcriptome predicted from the reference genome annotations.

Reference transcriptomes

For *Vitis vinifera*, the sequences of the transcriptome derived from the reference genome annotation were downloaded from the CRIBI genomes portal (http://genomes.cribi.unipd.it/DATA/V1/FASTA/V1_mrna.fa).

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The *Sorghum bicolor* reference transcriptome corresponds to the *Sorghum bicolor* v1.4 dataset available on the phytozome web site (http://www.phytozome.net/) at ftp://ftp.jgi-psf.org/pub/compgen/phytozome/v9.0/Sbicolor_v1.4/annotation/ (Sbicolor_79_transcript.fa).

BLAST analysis of contigs against the corresponding transcriptome predicted from the genome

For each contig, a BLASTN search was performed against the appropriate transcriptome reference. We only considered the first ten hits with an e-value of 1e-5. Hits were considered significant when (i) the alignment length (merging all high-scoring segment pairs) was at least 50% of the query sequence or at least 50% of the hit sequence, and (ii) sequence identity between query and hit was more than 90% across the alignment. This last threshold was chosen higher compared to Cahais *et al.* (2012) because contigs were here compared to the reference transcriptome of the same species.

Classification of BLAST results

Contigs were classified into seven categories, defined by the number and nature of significant BLAST hits. Based on Cahais *et al.* (2012) we distinguished the following cases:

a) Contigs with no significant hit were called 'No Hit'.

b) Contigs with a single significant hit and such that no other contig found the same hit. Within this category, contigs were called 'Full length' if the contig ⁄ hit alignment covered at least 90% of the hit sequence or 'Partial' otherwise.

c) Contigs with a single significant hit shared by other contigs.

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Within this category, when two contigs overlapped, if the length of the hit sequence to which both contig queries aligned covered at least 50% of the shortest of the two contigs, contigs were called “Allele”, whereas those showing no significant overlap with any other contigs were called “Fragment”.

d) Contigs with several significant hits, all specific to this contig.

When the hit sequences showed significant overlap, with the hit/hit alignment covering at least 50% of the shortest of the two hit sequences, contigs were called “Multi copies”. Whereas contigs whose hits showed no significant overlap were called “Chimera”.

e) Contigs not included in any of the above categories, involving more complex patterns.

If *m* contigs had *n* significant hits, and if *m* and *n* were equal, and when the *m* first hits of the *m* contigs were distinct from each other, contigs were called ‘Full length’ or ‘Fragment’, depending on the contig ⁄ hit alignment. In all other cases, contigs were called ‘Multi copies’.

Mapping of the contigs on the reference genome

We used Exonerate (Slater & Birney 2005) to perform mapping of the contigs on the reference genome. We used the following parameters: sizes of introns limited to 5,000 bp, model set to est2genome, and percent 50 and bestn 5 to keep the best five hits if their scores were at least 50% of the best hit. In order to easily compare the positions of our mapping with the predicted mRNAs, we used the parameter showtargetgff which gave the alignment in GFF format.

For the percentage of annotations mapped by contigs, we took into account the number of distinct annotations mapped. The annotations corresponded to the

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determined positions of mRNAs on the genome. The number of mapped annotations was determined by the tool IntersectBed from bedtools (Quinlan & Hall 2010).

BBMH (Best Blast Mutual Hits) comparison of transcriptomes

For *Solanum melongena,* the sequences of the transcriptome derived from annotation of the reference genome SME_r2.5.1 (Kazusa) was downloaded from ftp://ftp.kazusa.or.jp/pub/eggplant/SME_r2.5.1_cds.fa.gz, the published RNA-Seq transcriptome assembly (YanXu & al) from http://www.ncbi.nlm.nih.gov/Traces/wgs/?download=GBEF01.1.fsa_nt.gz, and the unigene set (Vegmark) from http://cse.naro.affrc.go.jp/fukuoka/EggEST/EggUnigene_16245.fasta.zip*.*

Within each transcriptome, we used CD-HIT (Fu *et al.* 2012) to remove highly similar sequences. To access pairs of similar sequences between compared data sets, Best Blast Mutual Hits (BBMH) analyses were computed with BLASTN and an e-value of 1e-10. Venn diagrams were created with jvenn (Bardou *et al.* 2014).

Functional Annotations

Contigs from the different assemblies were blasted as follows: firstly, they were compared to the Swissprot database. Queries with no hit were compared to the TrEMBL database. Then, queries with no hit against the TrEMBL database were compared to the NR database, and sequences still having no hit were compared against the NT database. We considered hits with an e-value of 1e^{-5} and only the best five hits were kept.

Sequences were translated using prot4EST (Wasmuth & Blaxter 2004). We provided it with the outputs of the proteic BLAST (against Swissprot, TrEMBL and NR).

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The BLAST output files were loaded into Blast2GO (Gotz *et al.* 2008) to perform Gene Ontology (GO) annotation and GO terms enrichment analysis. We used the three GO ontologies: molecular function, biological process, and cellular component. We categorized the enriched GO terms with CateGOrizer (Hu et al. 2008) using the GO_slim2 classification method and single occurrences count.

Results and Discussion

Validation of the assembly protocol

The performance of the different computational methods available for transcript reconstruction from RNA-Seq data depends on various criteria, including: the type of organism to be assembled; the preference given to either sensitivity or precision of the predictions; and the type of analysis to be performed with the reconstructed contigs (Steijger *et al.* 2013). Here, the criterion was to maximize production of accurately predicted gene sets that can be commonly found in the transcriptomes of different individuals of a species for intra-species analyses (e.g. SNP detection, population genomics). We followed the overall strategy of Cahais *et al.* (2012) because their protocol focused on optimizing the reconstruction of unambiguous correct cDNAs for comparative and population genomics purposes. We refined this assembly protocol by re-evaluating the utility of combining the assemblers Abyss (Simpson *et al.* 2009) and Cap3 (Huang & Madan 1999). We assessed and compared the quality of *de novo* Illumina short-read assemblies using data from two crops for which an annotated reference genome is available, grapevine (*Vitis vinifera subsp. vinifera*) (Jaillon *et al.* 2007) and sorghum (*Sorghum bicolor subsp. bicolor*) (Paterson *et al.* 2009) (see Comparison of assembly protocols in Materials and Methods). We chose these two species because they presented contrasting characteristics (e.g.: eudicot *vs* monocot,

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highly heterozygote outcrosser *vs* mainly homozygote selfer, GC-poor *vs* GC-rich), which allowed testing whether our protocol could be efficient for a wide genome diversity. Especially, highly heterozygote genomes, such as grapevine, should be more challenging than highly homozygote ones, and it is important to know whether protocols should be adapted or not to the expected heterozygosity level. Cahais *et al.* (2012) used reads from multiple individuals to increase the number of assembled contigs as some genes can be lowly or not expressed in some individuals. However, to evaluate the trade-off between maximizing the number of transcripts that can be assembled and optimizing the quality of the assembly by minimizing the complexity of the pool of reads to be assembled, we also compared assembly protocols using reads from multiple accessions or genotypes from the same species (*All Individuals*) *versus* protocols using reads from only one individual (*One Individual*) (Table 2). For the *One Individual* assembly, we chose the individual with the largest number of reads e.g. EC4 for *Sorghum bicolor* and VC2 for *Vitis vinifera*.

Quantitative assessment of the assemblies

For *Sorghum bicolor*, the number of transcripts predicted from the genome sequence (29,448), was equivalent to the number of contigs produced by the assembly with Abyss One Individual EC4 (27,733), and equivalent to Abyss One Individual EC4 combined with one or two steps of assembly with Cap3 (26,686 and 26,591 respectively). Using reads from all the individuals and an assembly with Abyss (A), Abyss/Cap3 (A/C), or Abyss/Cap3/Cap3 (A/C/C), the number of contigs roughly doubled (62,341, 58,441 and 54,974 respectively). With Trinity EC4, the number of contigs tended towards 70,000 contigs (Figure 1A).

For *Vitis vinifera*, we confirmed that the number of transcripts predicted from the genomic sequence, Genome (29,971), was close to the number of contigs produced by the assembly with Abyss One Individual VC2 (26,685) and Abyss One Individual VC2

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combined with one or two steps of assembly with Cap3 (24,315 and 24,015 respectively). With Trinity VC2, the number of contigs roughly doubled (48,934). Using all the individuals and Abyss, Abyss/Cap3, and Abyss/Cap3/Cap3 the number of contigs increased 3-4 fold (85,420, 87,125, and 80,982 respectively) (Figure 1B).

For both *Sorghum bicolor* and *Vitis vinifera*, the N50 and the mean contig length were higher and closer to those predicted from the genomic sequence (Genome) with all the combinations using one individual (EC4 and VC2) compared to the combinations using all the individuals (Figures 1C and 1D). No significant difference in N50 and mean contig length was detected between assembly with Trinity and the different combinations with Abyss using one individual (Figures 1C and 1D).

The percentage of contigs mapped on the genome was around 80-90% for *Sorghum bicolor* and 90-100% for *Vitis vinifera* (Figure S1). The percentage of predicted genes from the genome sequences mapped by the contigs was about 60% for the *One Individual* assemblies, and about 70-80% for the assemblies using Trinity and for the *All Individuals* assemblies (Figures 2A and 2B).

Representativeness of the assemblies

For *Sorghum bicolor*, 85-90% of the total reads from 10 individuals mapped to the assemblies built with Trinity, A/C EC4, A/C/C EC4, and A/C/C EC_All (Figure 3A). This percentage slightly decreased with A EC4 (77%), A/C EC_All (81%), and substantially decreased with A EC_All (53%).

For *Vitis vinifera*, 85-90% of the total reads from 12 individuals mapped to the assembly built with Trinity, A/C VC2, and A/C/C VC2 (Figure 3B). This percentage slightly decreased with A VC2 (77%), A/C VC_All (67%), A/C/C VC_All (82%), and substantially decreased with A VC_All (30%).

For both *Sorghum bicolor* and *Vitis vinifera*, a higher percentage of mapped reads was obtained when the *One Individual* assembly (produced using either Trinity, A/C or

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A/C/C) was used as reference compared to mapping carried out against CDS transcript sequences predicted from the genome sequence (Figures 3).

The percentage of reads mapped or correctly mapped to the built transcriptome were quite similar, depending on the different individuals. Indeed, we observed less than an 11% difference between individuals for both *Sorghum bicolor* and *Vitis vinifera* (Table S1).

For *Sorghum bicolor*, around 40% of the contigs from the A, A/C and A/C/C One Individual EC4 assemblies were mapped at an average coverage of 8X by reads from the 10 individuals. This percentage decreased notably for the Trinity (15%), A EC_All (23%), A/C EC_All (26%) and A/C/C EC_All (25%) assemblies. For *Vitis vinifera*, around 66-67% of the contigs from the A, A/C and A/C/C One Individual VC2 assemblies were mapped at an average coverage of 8X by reads from the 12 individuals. This percentage decreased notably for the Trinity (38%), A VC_All (26%), A/C VC_All (37%) and A/C/C VC_All (39%) assemblies (Table S2).

Categorization of the predicted contigs

We applied the categorization of contigs used in Cahais *et al.* (2012) in order to define the assembly protocol minimizing the probability of having erroneous predictions such as chimeras, unassembled alleles and assembled paralogues, which would mislead gene orthology prediction and SNP detection. This categorization was based on the number and the nature of significant BLAST hits of the contigs against a transcriptome predicted from the reference genome annotation (see Classification of BLAST results in Materials and Methods).

The ratio of non-problematic contigs (Full length+Partial+Fragment) was highest with A/C and A/C/C One Individual (>60% for *Sorghum bicolor* and *Vitis vinifera*). A/C/C One Individual gave similar results to A/C One Individual: 62 % of non-problematic contigs for A/C EC4 and A/C/C EC4, 64% for A/C VC2 and A/C/C VC2. We slightly

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improved the percentage of non-problematic contigs by combining Abyss with Cap3 compared to Abyss alone with 60% for A EC4 and A VC2. The percentage of non-problematic contigs decreased for Trinity and All Individuals: 27% for T EC4 and 32% for T VC2, 38% for A EC_ALL and 30% for A VC_ALL, 41% for A/C EC_ALL and A/C/C EC_ALL, 37% for A/C VC_ALL and A/C/C VC_ALL (Figures 4A and 4B). With Trinity's assembly using reads from all individuals, we confirmed that the percentage of non-problematic contigs substantially decreased (7% for *Sorghum bicolor* and 4 % for *Vitis vinifera*) (Table S3). To assess the impact of isoforms on our classification of contigs, we filtered out isoforms predicted by the Butterfly module of Trinity. From each group of predicted isoforms, we kept the longest one and then we recalculated the classification of contigs. We found that the percentage of non-problematic contigs is still low for Trinity after filtering isoforms: for *Sorghum bicolor* All Individuals and One individual (15% and 24% respectively), and for *Vitis vinifera* All Individuals and One individual (12% and 28 % respectively) (Table S4).

We checked whether filters on RPKM could change our results. We compared the percentage of non-problematic contigs for the T EC4, A/C EC4 and A/C EC_ALL assemblies using different filters on RPKM. The A/C EC4 assembly always gave the best score of non-problematic contigs with values greater than 60% (Figure 5).

Conclusions of the assemblies' comparison and choice of the optimal transcriptome assembly strategy

We found that the N50 and mean length of assemblies were higher and closer to those predicted from the genome sequence with all the One Individual combinations compared to the All Individuals combinations (Figures 1C and 1D). The percentage of reads from all the individuals that were mapped or correctly mapped to the One Individual assemblies was greater than the percentage of reads mapped to the All

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Individuals contigs (Figures 3). It is worth noting that the One Individual assemblies produced transcriptome references that were representative of all the sequenced individuals, probably due to fewer erroneous contigs.

We also found that the One Individual assemblies produced by Abyss combined with one step of Cap3 gave the best ratio of non-problematic contigs (Figures 4). We checked that applying filters on read depth of coverage did not notably affect the evaluation results (Figure 5). Lastly, our conclusion was that the optimal protocol was Abyss combined with one step of Cap3, using reads from only one sequenced individual. This protocol led to 95% (*Sorghum bicolor*) and 97% (*Vitis vinifera*) of the contigs being mapped on the corresponding reference genome sequence. We favoured this protocol because our aim was to provide reference transcriptomes that could be useful for further comparative and population genomic analyses, even at the expense of transcriptome exhaustivity. It is also interesting that the same protocol performed the best (according to our criteria) for the two tested genomes despite their contrasting characteristics, especially their different heterozygosity level. This is also another advantage to have a protocol robust to change in genome characteristics. Nevertheless, for other purposes a more exhaustive and detailed representation of the transcriptome might be preferred. In such cases, the Trinity protocol appears to be more suitable, especially for highly heterozygous species.

Assembly of 26 new transcriptomes

We assembled 26 new transcriptomes of cultivated plants and close relative species (listed in Table 1) using the Abyss-Cap3-One Individual protocol. The N50, mean contig length, and percentages of aligned and annotated contigs (Table 3) were comparable to those of other recently published transcriptomes (Bhardwaj *et al.* 2013; Kudapa *et al.* 2014; Munoz-Merida *et al.* 2013; Zhang *et al.* 2013). Using our assembly

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protocol, contig numbers ranged from 19,811 in *Herrania nitida* (a relative of cocoa) to 59,792 in *Phillyrea angustifolia* (a relative of olive tree) (average = 32,755).

Some of our new assemblies (eggplant, pepper, oil palm, olive tree) already have corresponding reference genomes or transcriptomes published. As an example of comparison, we assessed overlaps (see BBMH comparison of transcriptomes in Materials and Methods) between our eggplant assembly and three types of available data sources: the transcripts predicted from the eggplant draft genome SME_r2.5.1 (Hirakawa *et al.* 2014), a previously published transcriptome assembly from RNA-Seq (Yang *et al.* 2014), and with a unigene set produced by cDNA sequencing (Vegmark) (Fukuoka *et al.* 2010). We found that around 81% (21,574 for a total of 26,752 non redundant sequences) of our eggplant contigs have Best Blast Mutual Hit (BBMH) to at least one of the queried data sources. A core of 8,820 sequences (33%) were commonly found in the three data sets (Figure 6). The published eggplant RNA-Seq assembly (YanXu & al) showed more BBMH (18,703) compared to the transcripts predicted from the draft genome SME_r2.5.1 (15,707) and to the unigene set (11,865). This is probably correlated to a more complete view of the transcriptome provided by RNA-Seq.

Functional annotations

The contigs of the 26 reference transcriptomes were annotated using BLAST, prot4EST (Wasmuth & Blaxter 2004), and Blast2GO (Gotz *et al.* 2008). The 26 transcriptomes had more than 60% of the contigs having BLAST hits, and 24 transcriptomes had more than 70% of aligned contigs. For 21 transcriptomes, more than 50% of the contigs had a BLAST hit against the high quality manually curated annotations of the Swiss-Prot database. In addition, 25 transcriptomes had more than 40% of the contigs annotated with Gene Ontology terms (Table 2).

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As an example of biological insights that may be provided by our resource, we assessed GO term enrichment in the group of nine transcriptomes of the Poaceae family (*Triticum monococcum, Eremopyrum bonaepartis, Taeniatherum caput-medusae, Sorghum brachypodum, Sorghum bicolor, Pennisetum glaucum, Pennisetum polystachion, Pennisetum alopecuroides,* and *Digitaria exilis*), using the Fisher's Exact Test function implemented in Blast2GO (Gotz et al. 2008). Each transcriptome of the Poaceae group was compared to all other transcriptomes of the group. We filtered with a FDR (False Discovery Rate) value of 0.05. The transcriptome of *Sorghum bicolor* showed a large number of over-represented terms (8,761) with a slight overrepresentation of terms associated with metabolism (14%) (Figure S2). The transcriptome of *Sorghum brachypodum* showed a smaller number of GO terms over-represented (35) (Table S5) with a clear overrepresentation of terms associated with metabolism and binding (Figure 7). The other Poaceae transcriptomes have no more than 5 over-represented GO terms. Note that in this simple analysis “fragment” contigs count twice (or more) for a GO category. This simple analysis could thus be affected by the different species having different proportions of contigs categories. A more detailed analysis of the corresponding contigs and related annotations might lead to new insights into the evolution and/or expression of some specific gene families in *Sorghum brachypodum* compared to other Poaceae species.

For the 26 new transcriptomes, all the sequences (FASTA format) and annotations (GFF3 format), together with BLAST, prot4EST, and Blast2GO results are freely available at http://arcad-bioinformatics.southgreen.fr/. These transcriptomes and their annotations represent a significant resource for large scale analyses of relationships between the evolution, diversity and function of gene families.

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Data Accessibility

Raw data were deposited in the NCBI Sequence Read Archive (SRA) under BioProject PRJNA326055.

Sequences assemblies (FASTA), annotations (GFF, Blast XML, Blast2GO .annot, FASTA prot4EST translations): http://arcad-bioinformatics.southgreen.fr.

Scripts used for the analyses: https://github.com/SouthGreenPlatform/arcad-hts.

Author Contributions

Sylvain Glémin, Jacques David, Gautier Sarah and Manuel Ruiz designed research. Gautier Sarah, Felix Homa, Stéphanie Pointet, Sandy Contreras, François Sabot and Benoit Nabholz developed scripts and performed bioinformatic analyses. Sylvain Glémin, Gautier Sarah and Manuel Ruiz wrote the paper. Sylvain Santoni, François Sabot, Laure Sauné, Morgane Ardisson, Nathalie Chantret, Christopher Sauvage, James Tregear, Cyril Jourda, David Pot, Yves Vigouroux, Hana Chair, Nora Scarcelli,

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Figure legends

Figure 1. Number of contigs, N50 and mean contig length obtained by the different assembly protocols. The number of contigs increases with Trinity alone and All Individuals (**A**. *Sorghum bicolor*, **B**. *Vitis vinifera*), the N50 and mean length decreased with All Individuals (**C**. *Sorghum bicolor*, **D**. *Vitis vinifera*).

T, Trinity; A, Abyss; C, Cap3; Genome, transcripts predicted from the genome sequence; EC4, one individual *Sorghum bicolor* EC4; VC2,one individual *Vitis vinifera* VC2; EC_ALL, 10 individuals *Sorghum bicolor*; VC_ALL, 12 individuals *Vitis vinifera*.

Figure 2. Percentage of predicted genes mapped by the contigs obtained by the different assembly protocols. The percentage of predicted genes from the *Sorghum bicolor* and *Vitis vinifera* genome sequences that showed an overlap with the contigs was about 60% for One Individual, and 70-80% for Trinity alone and All Individuals: (**A**) *Sorghum bicolor* and (**B**) *Vitis vinifera*.

T, Trinity; A, Abyss; C, Cap3; EC4, one individual *Sorghum bicolor* EC4; VC2,one individual *Vitis vinifera* VC2; EC_ALL, 10 individuals *Sorghum bicolor*; VC_ALL, 12 individuals *Vitis vinifera*.

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Figure 3. Percentage of the total reads mapped to the assemblies built with the different protocols. The assemblies with One Individual were the most representative of all the individuals: (**A**) *Sorghum bicolor* and (**B**) *Vitis vinifera*.

T, Trinity; A, Abyss; C, Cap3; Genome, transcripts predicted from the genome sequence; EC4, one individual *Sorghum bicolor* EC4; VC2,one individual *Vitis vinifera* VC2; EC_ALL, 10 individuals *Sorghum bicolor*; VC_ALL, 12 individuals *Vitis vinifera*.

Figure 4. Ratio of non-problematic contigs obtained by the different assembly protocols. The ratio of non-problematic predicted contigs (Full length+Partial+Fragment) was better with AC and ACC One Individual: (**A**) *Sorghum bicolor* and (**B**) *Vitis vinifera*.

T, Trinity; A, Abyss; C, Cap3; EC4, one individual *Sorghum bicolor* EC4; VC2,one individual *Vitis vinifera* VC2; EC_ALL, 10 individuals *Sorghum bicolor*; VC_ALL, 12 individuals *Vitis vinifera*.

Figure 5. Effect of RPKM filters on the ratio of non-problematic predicted contigs. The transcriptome assembly using Abyss with one step of Cap3 (A/C) and reads from the EC4 *Sorghum bicolor* individual (A/C EC4) maintained the best ratio of non-problematic predicted contigs (% of contigs from the categories Full length+Partial+Fragment) using different filters on RPKM : 0,1,2,3,4 (Reads per Kilobase per Million Reads).

T, Trinity; A, Abyss; C, Cap3; EC4, one individual *Sorghum bicolor* EC4; EC_ALL, 10 individuals *Sorghum bicolor*.

Figure 6. Number of Best Blast Mutual Hits of our eggplant assembly with transcripts predicted from the draft genome SME_r2.5.1 (Kazusa), with a previously published transcriptome assembly from RNA-Seq (YanXu & al), and with a unigene set (Vegmark). The Venn diagram displays overlaps between the three data sets. Charts

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showing the list size (number of BBMH) and intersection size repartition are located underneath the diagram.

Figure 7. Main GO categories of the over-represented GO terms for *Sorghum brachypodum* contigs compared to other transcriptomes of the Poaceae family. The reference transcriptomes used for the Poaceae family were *Triticum monococcum, Eremopyrum bonaepartis, Taeniatherum caput-medusae, Pennisetum glaucum, Pennisetum polystachion, Pennisetum alopecuroides, Digitaria exilis, Sorghum bicolor.*

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Table 1. Summary of sequenced individuals of cultivated plants and close relative species

Family	Taxon	Crop name	Life span	Mating system	Number of chromosomes	Close relative crop	Accession	Germplasm source	Sources of RNA[3]
Arecaceae	*Elaeis guineensis*	Oil palm	Perennial	Allogamy	2n=32		LM18293-Eg02a	INRAB, Centre de Recherches Agricoles Plantes Pérennes, Station Pobè, Bénin	80% inflorescence, 20% leaves
	Mauritia flexuosa		Perennial	Allogamy	2n=30	Oil palm	Wild individual (male), nr. Kourou, French Guiana	Field collection, French Guiana	80% inflorescence, 20% leaves
Dioscoreaceae	*Dioscorea rotundata*	Yam	Annual	Allogamy	2n=40		CR4885	IRD Montpellier, France	80% inflorescence, 20% leaves
	Dioscorea alata		Annual	Allogamy	2n=40	Yam		CIRAD collection, Guadeloupe, French West Indies	80% inflorescence, 20% leaves
	Dioscorea trifida		Annual	Allogamy	2n=40	Yam		CRB-PT (Centre des Ressources Biologiques_Plantes Tropicales), Guadeloupe, French West Indies	80% inflorescence, 20% leaves
Fabaceae	*Medicago sativa*	Alfalfa	Perennial	Allogamy	2n = 4x = 32		Gongnond	INRA collection Biological Ressources Center Medicago Montpellier	80% inflorescence, 20% leaves
	Medicago		Perennial	Allogamy	2n = 16	Alfalfa	"carnon"	INRA collection	80% infloresc

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	marina							Biological Ressources Center Medicago Montpellier	ence, 20% leaves
Malvaceae	*Herrania nitida*		Perennial	Unknown	2n=20	Cocoa	C4X6Y9	International Cocoa Genebank, Trinidad UWI Campus St Augustine Trinidad and Tobago	80% inflorescence, 20% leaves
	Theobroma speciosa	Cacaui	Perennial	Unknown	2n=20	Cocoa	Bord6B	International Cocoa Genebank, Trinidad UCRS Centeno Trinidad and Tobago	80% inflorescence, 20% leaves
Musaceae	*Musa balbisiana*		Perennial	Allogamy	2n = 22	Banana	'Pisang Klutuk Wulung' (PT-BA-00302)	CIRAD collection Guadeloupe, CRB Plantes tropicales	65% inflorescence, 15% leaves, 20% fruits
	Musa beccarii		Perennial	Allogamy	2n = 20	Banana	Musa beccarii (PT-BA-00221)	CIRAD collection Guadeloupe, CRB Plantes tropicales	65% inflorescence, 15% leaves, 20% fruits
Oleaceae	*Olea europaea subsp. europaea*	Olive tree	Perennial	Allogamy	2n = 46		Arbequina (M3-29)	INRA collection (UE DiaScope, Montpellier)	80% inflorescence, 20% leaves
	Olea europaea subsp. cuspidata		Perennial	Allogamy	2n = 46	Olive tree	Graham Stawn n° 3	CEFE collection (CNRS, Montpellier)	80% inflorescence, 20% leaves
	Phillyrea angustifolia		Perennial	Allogamy	2n = 46	Olive tree		CEFE collection (CNRS, Montpellier)	80% inflorescence, 20% leaves
Poaceae	*Triticum monococcum*	Einkorn whe	Annual	Autogamy	2n = 14		ID 259	Max Planck Institut für Züchtungsfo	80% inflorescence,

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		at						rschung, Köln, Germany	20% leaves
	Eremopyrum bonaepartis		Annual	Autogamy	2n = 14	Einkorn wheat	PI203442	National Small Grain Collection, Aberdeen, Idaho, USA	65% inflorescence, 15% leaves, 20% grains
	Taeniatherum caput-medusae		Annual	Autogamy	2n =14	Einkorn wheat	PI577708	National Small Grain Collection, Aberdeen, Idaho, USA	65% inflorescence, 15% leaves, 20% grains
	Sorghum brachypodum		Annual	Autogamy[1]	2n=10[2]	Sorghum	JC2125	Australian Tropical Crops and Forages Collection (QDPI)	65% inflorescence, 15% leaves, 20% grains
	Pennisetum glaucum	Pearl millet	Annual	Allogamy	2n=14		PE08038	IRD Montpellier, France	65% inflorescence, 15% leaves, 20% grains
	Pennisetum polystachion		Annual	Allogamy	2n=18	Pearl millet	O914	IRD Montpellier, France	80% inflorescence, 20% leaves
	Pennisetum alopecuroides		Annual	Allogamy	2n=18	Pearl millet	A1	IRD Montpellier, France	80% inflorescence, 20% leaves
	Digitaria exilis	Fonio	Annual	Autogamy	2n=36		CM 07892	IRD Montpellier, France	80% inflorescence, 20% leaves
Solanaceae	*Solanum melongena*	Eggplant	Annual	Autogamy	2n=24	Tomato	MM738 (Netherlands)	INRA GAFL, France	80% inflorescence, 20% leaves
	Capsicum annuum	Pepper	Annual	Autogamy	2n=24	Tomato	PM659 (North West	INRA, GAFL, France	65% inflorescence,

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							India – Punjab)		15% leaves, 20% fruits
Vitaceae	*Vitis romanetii*		Perennial	Allogamy	2n=38	Grapevine	8065Mtp2	INRA - Domaine de Vassal, France[4]	65% inflorescence, 15% leaves, 20% fruits
	Vitis riparia		Perennial	Allogamy	2n=38	Grapevine	9006Mtp1	INRA - Domaine de Vassal, France[4]	80% inflorescence, 20% leaves

[1] There is no clear information regarding the reproductive behaviour of this species. It is quite likely that it is autogamous but with a significant proportion of allogamy (even some cultivated and wild sorghum display significant proportions of allogamy, up to 20% in some cases for cultivated sorghum)

[2] Cultivated sorghum is 2n=20

[3] Leave means young leave, fruit means immature fruit, and grain means immature grain

[4] The grape biological materials were provided by the Center for Grape Genetic Resources, at the INRA “Domain de Vassal”, Marseillan Plage, France: http://bioweb.ensam.inra.fr/collections_vigne/Home.php?l=EN

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Table 2. Summary of the sequenced individuals in sorghum (*Sorghum bicolor subsp. bicolor*) and grapevine (*Vitis vinifera subsp.vinifera*)

Sorghum bicolor subsp. bicolor		*Vitis vinifera subsp. vinifera*	
Study code	**Accession**[1]	**Study code**	**Accession**[2]
EC1	SSM1049	VC1	Lameiro (0Mtp636)
EC2	IS29876	VC2	Morenoa (0Mtp739)
EC3	IS30436	VC3	Verdelho tinto (0Mtp1176)
EC4	SSM1123	VC4	Clairette blanche (26Mtp2)
EC5	IS6193	VC5	Mondeuse blanche (176Mtp1)
EC6	SSM973	VC6	Savagnin 77 (Savagnin blanc) (257Mtp16)
EC7	IS14317	VC7	Katta kourgan (556Mtp2)
EC8	IS29407	VC8	Chasselas 3 (585Mtp62)
EC9	Tigne de Dangalma (SSM1057)	VC9	Zinfandel (Primitivo) (1277Mtp6)
EC10	IS26554	VC10	Araklinos (1805Mtp1)
		VC11	Tita caprei (Tsitsa Kaprei) (2471Mtp1)
		VC12	Labrusco noir (2953Mtp1)

[12]The sorghum biological materials were provided by the the CIRAD, Laboratoire de Semences et de Ressources Génétiques, France: LSRG, http://golo.cirad.fr

[2]The grape biological materials were provided by the Center for Grape Genetic Resources, at

the INRA “Domain de Vassal”, Marseillan Plage, France:

http://bioweb.ensam.inra.fr/collections_vigne/Home.php?l=EN

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cle

Table 3. Summary of the assemblies and annotations

🟧 % contigs with BLAST SP+Tr+NR > 🟠 70%, % contigs with BLAST SP > 🟨 50%, % contigs with blast2GO > 40%

Family	Taxon	Crop name	Number of contigs	N50	Mean length	% BLAST SP+Tr+NR	% BLAST SP	% Blast2GO
Arecaceae	*Elaeis guineensis*	Oil palm	26,791	1,468	1,005	77	57	50
	Mauritia flexuosa		46,660	1,386	927	62	46	39
Dioscoreaceae	*Dioscorea rotundata*	Yam	30,551	1,626	1,137	77	58	48
	Dioscorea alata		26,681	1,525	1,069	75	58	48
	Dioscorea trifida		29,448	1,405	971	72	55	46
Fabaceae	*Medicago sativa*	Alfalfa	30,358	1,258	898	85	59	48
	Medicago marina		30,009	1,638	1,182	86	60	41
Malvaceae	*Herrania nitida*		19,811	1,067	835	85	62	53
	Theobroma speciosa		22,440	1,222	916	82	61	52
Musaceae	*Musa balbisiana*		29,356	1,060	791	90	64	54

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	Musa beccarii		50,365	1,203	848	76	53	44
Oleaceae	*Olea europaea subsp. europaea*	Olive tree	45,389	1 094	794	78	57	46
	Olea europaea subsp. cuspidata		37,870	1,390	980	82	61	49
	Phillyrea angustifolia		59,792	1,024	781	71	54	43
Poaceae	*Triticum monococcum*	Einkorn wheat	33,381	1,492	1,027	67	46	42
	Eremopyrum bonaepartis		34,255	1,406	974	70	47	43
	Taeniatherum caput-medusae		32,786	1,450	993	73	50	45
	Sorghum brachypodum		25,814	995	749	84	56	54
	Pennisetum glaucum	Pearl millet	24,618	1,422	1,021	82	58	53
	Pennisetum polystachion		37,117	1,332	940	73	49	44
	Pennisetum alopecuroides		34,073	1,442	1,001	70	48	43
	Digitaria exilis	Fonio	37,326	1,318	932	76	51	48
Solanaceae	*Solanum melongena*	Eggplant	26,807	1,649	1,162	85	62	49
	Capsicum annuum	Pepper	38,170	1,68	1,176	74	52	42

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				7				
Vitaceae	*Vitis romanetii*		30,326	1,767	1,239	80	57	49
	Vitis riparia		30,572	1,729	1,203	81	57	49

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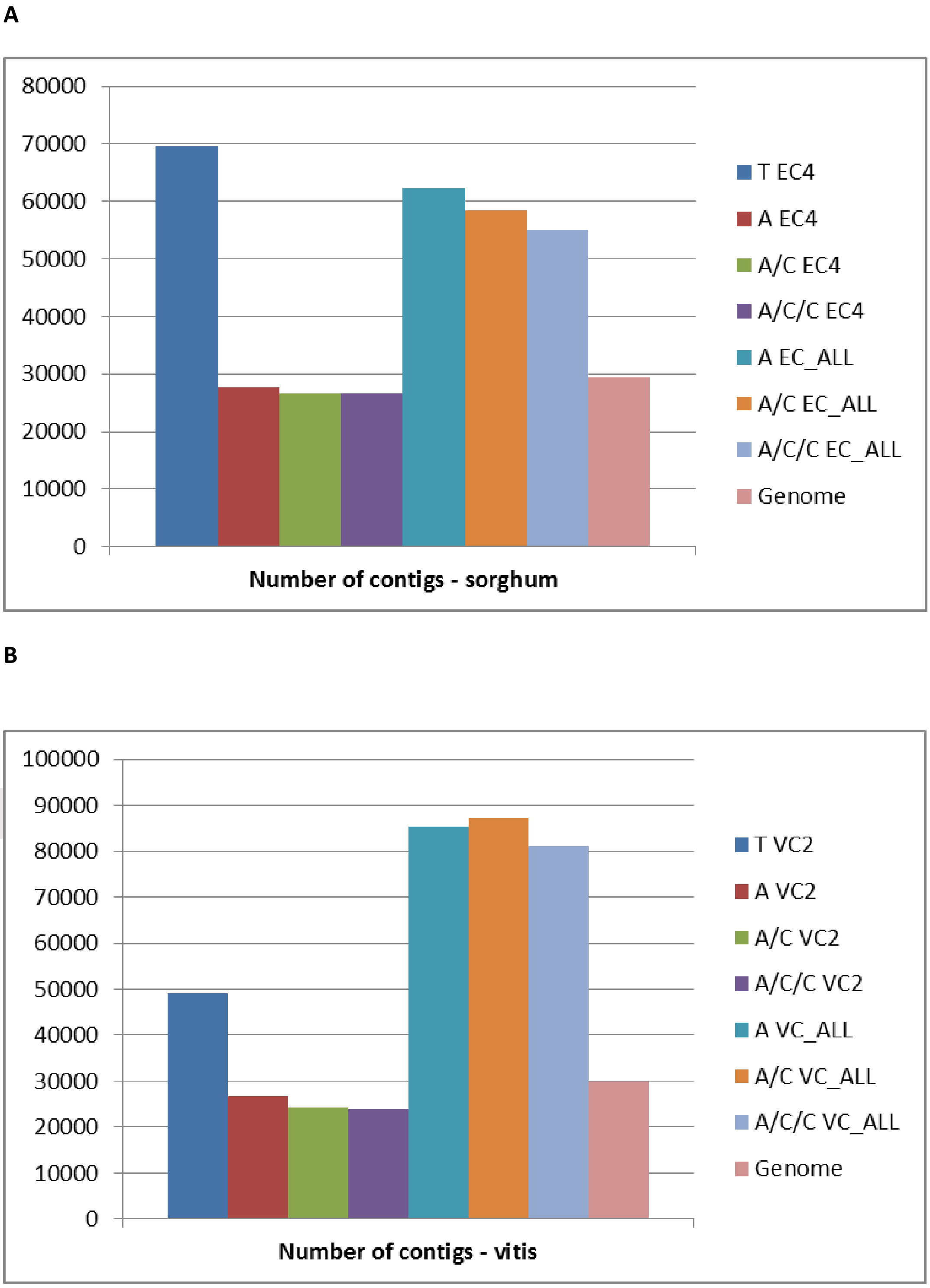
Figure 1. Number of contigs, N50 and mean contig length obtained by the different assembly protocols. The number of contigs increases with Trinity alone and All Individuals (**A**. *Sorghum bicolor*, **B**. *Vitis vinifera*), the N50 and mean length decreased with All Individuals (**C**. *Sorghum bicolor*, **D**. *Vitis vinifera*).
T, Trinity; A, Abyss; C, Cap3; Genome, transcripts predicted from the genome sequence; EC4, one individual *Sorghum bicolor* EC4; VC2,one individual *Vitis vinifera* VC2; EC_ALL, 10 individuals *Sorghum bicolor*; VC_ALL, 12 individuals *Vitis vinifera*.



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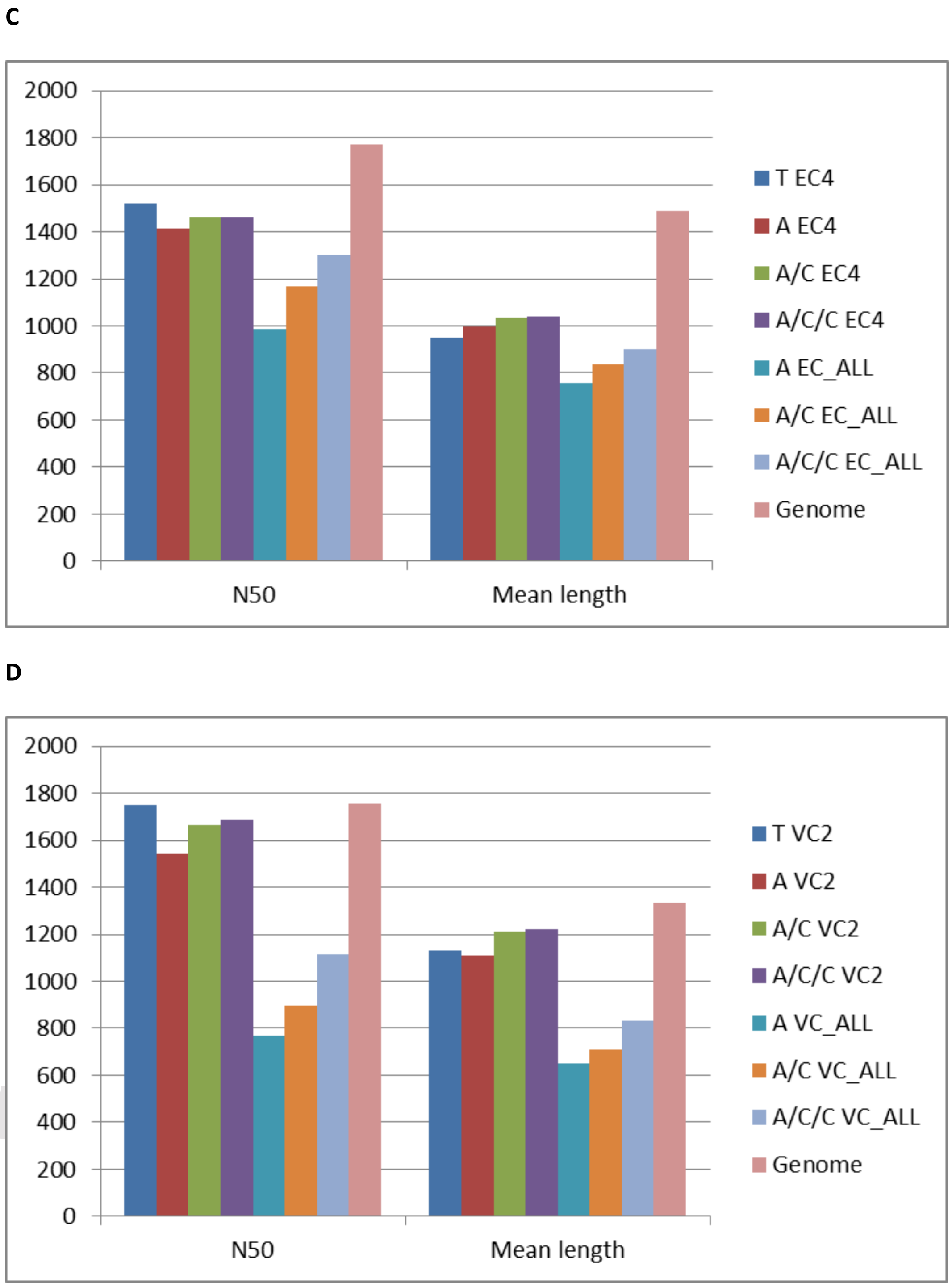

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Figure 2. Percentage of predicted genes mapped by the contigs obtained by the different assembly protocols. The percentage of predicted genes from the *Sorghum bicolor* and *Vitis vinifera* genome sequences that showed an overlap with the contigs was about 60% for One Individual, and 70-80% for Trinity alone and All Individuals: (**A**) *Sorghum bicolor* and (**B**) *Vitis vinifera*.

T, Trinity; A, Abyss; C, Cap3; EC4, one individual *Sorghum bicolor* EC4; VC2,one individual *Vitis vinifera* VC2; EC_ALL, 10 individuals *Sorghum bicolor*; VC_ALL, 12 individuals *Vitis vinifera*.

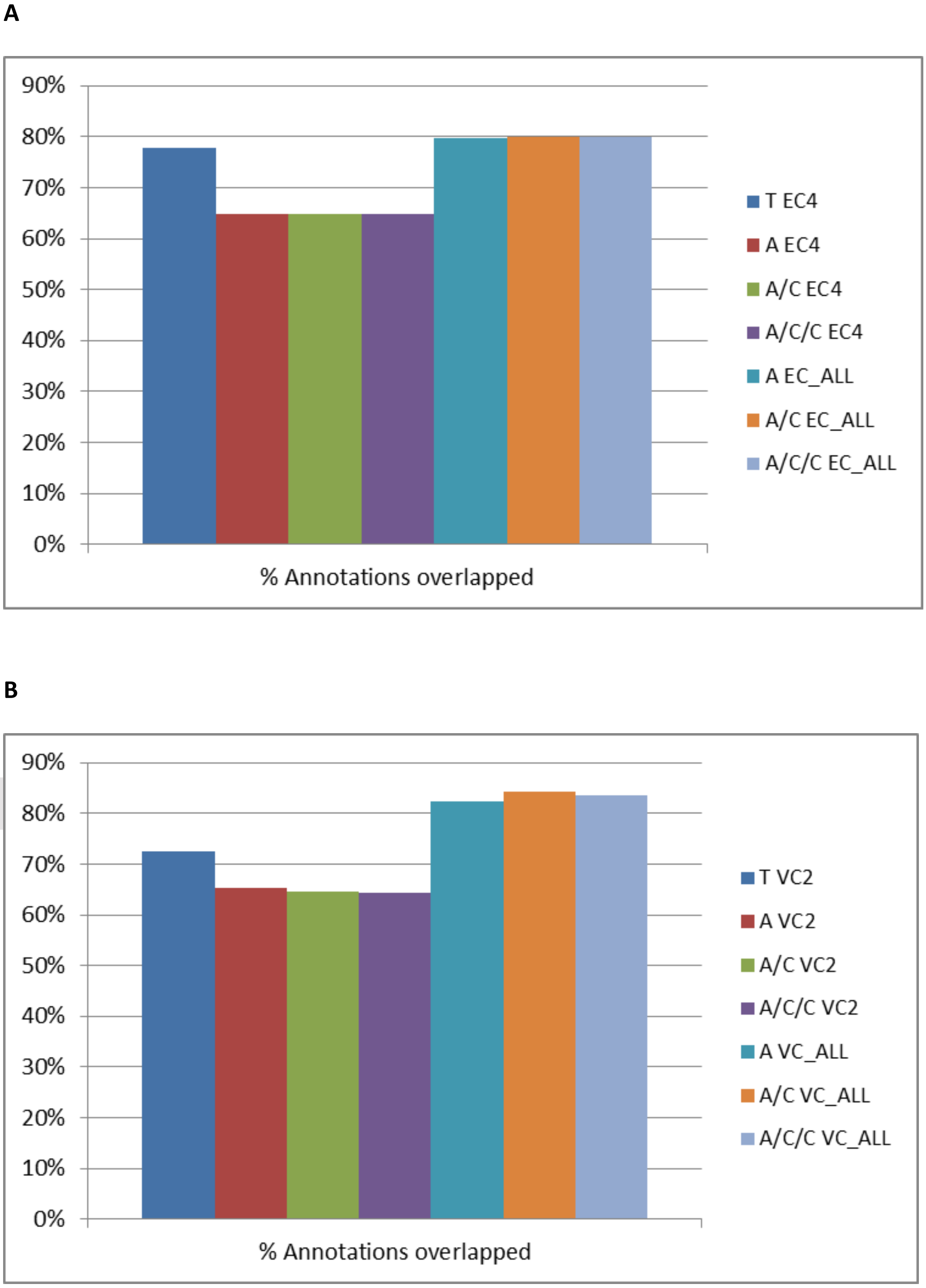

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Figure 3. Percentage of the total reads mapped to the assemblies built with the different protocols. The assemblies with One Individual were the most representative of all the individuals: (**A**) *Sorghum bicolor* and (**B**) *Vitis vinifera*.
T, Trinity; A, Abyss; C, Cap3; Genome, transcripts predicted from the genome sequence; EC4, one individual *Sorghum bicolor* EC4; VC2,one individual *Vitis vinifera* VC2; EC_ALL, 10 individuals *Sorghum bicolor*; VC_ALL, 12 individuals *Vitis vinifera*.

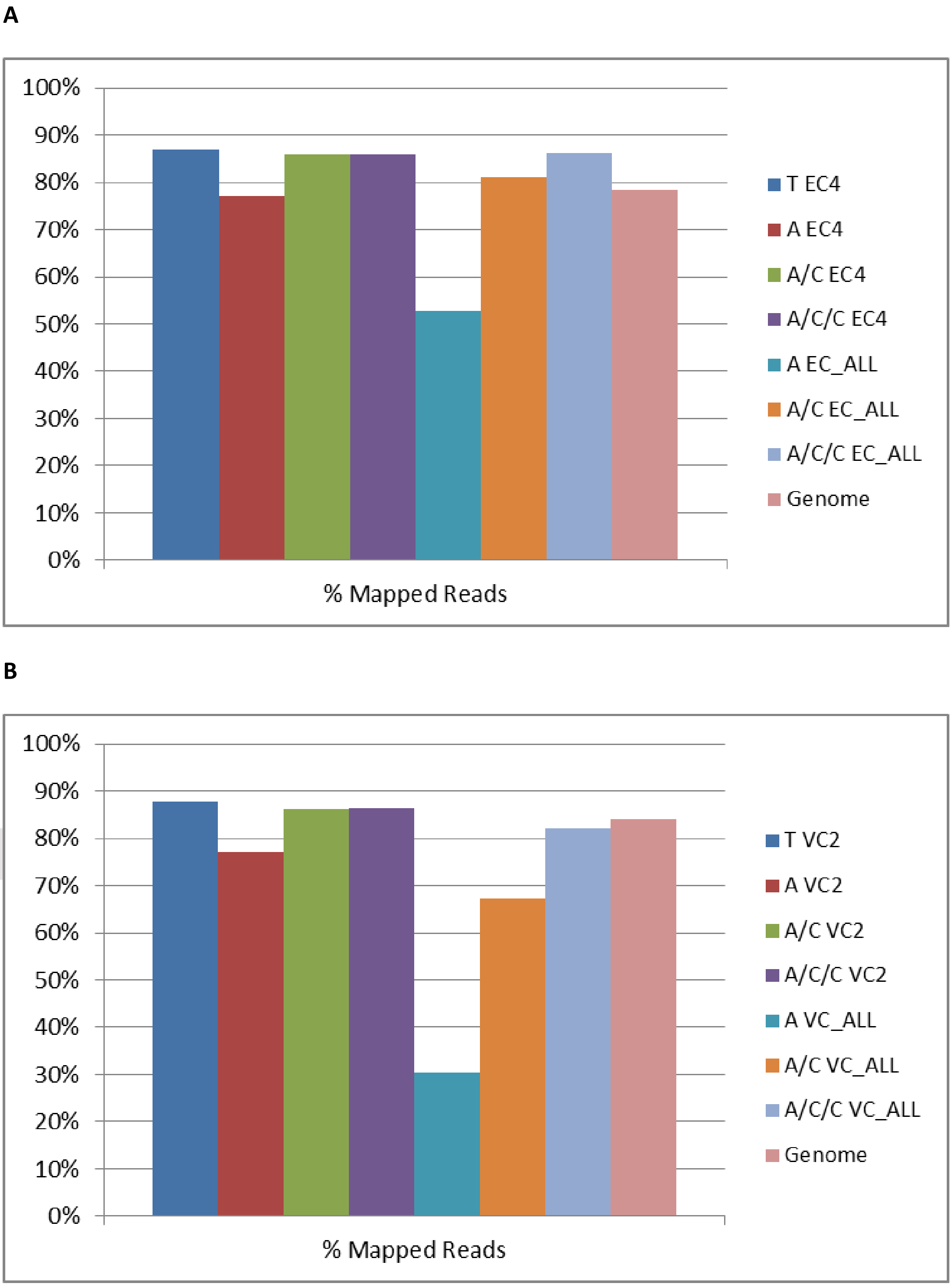

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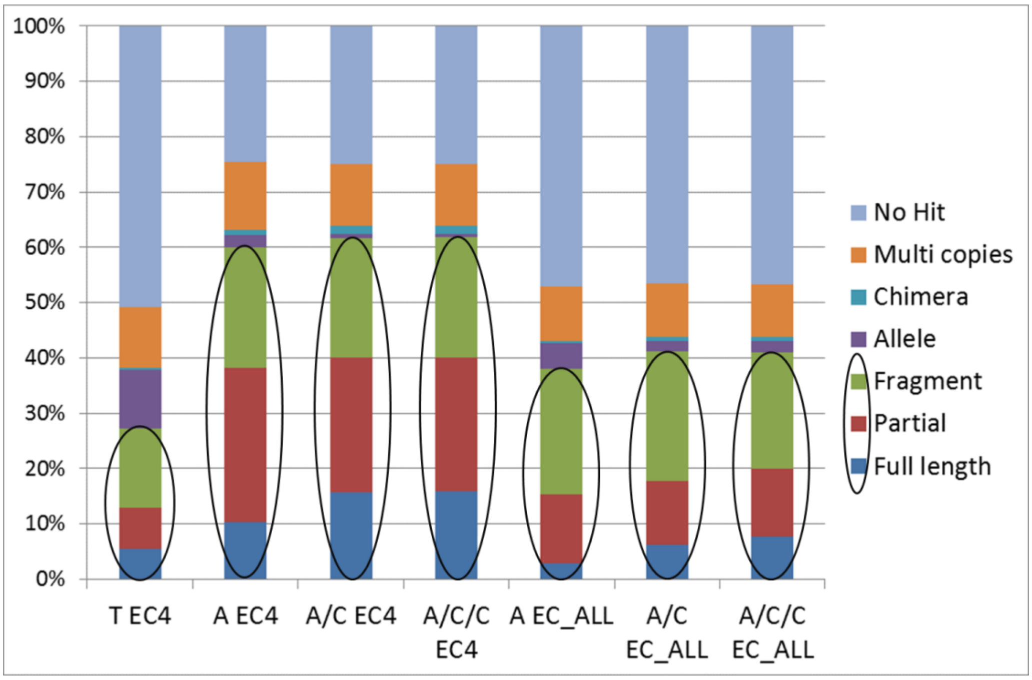
Figure 4. Ratio of non-problematic contigs obtained by the different assembly protocols. The ratio of non-problematic predicted contigs (Full length+Partial+Fragment) was better with AC and ACC One Individual: (**A**) *Sorghum bicolor* and (**B**) *Vitis vinifera*.
T, Trinity; A, Abyss; C, Cap3; EC4, one individual *Sorghum bicolor* EC4; VC2,one individual *Vitis vinifera* VC2; EC_ALL, 10 individuals *Sorghum bicolor*; VC_ALL, 12 individuals *Vitis vinifera*.

A



B

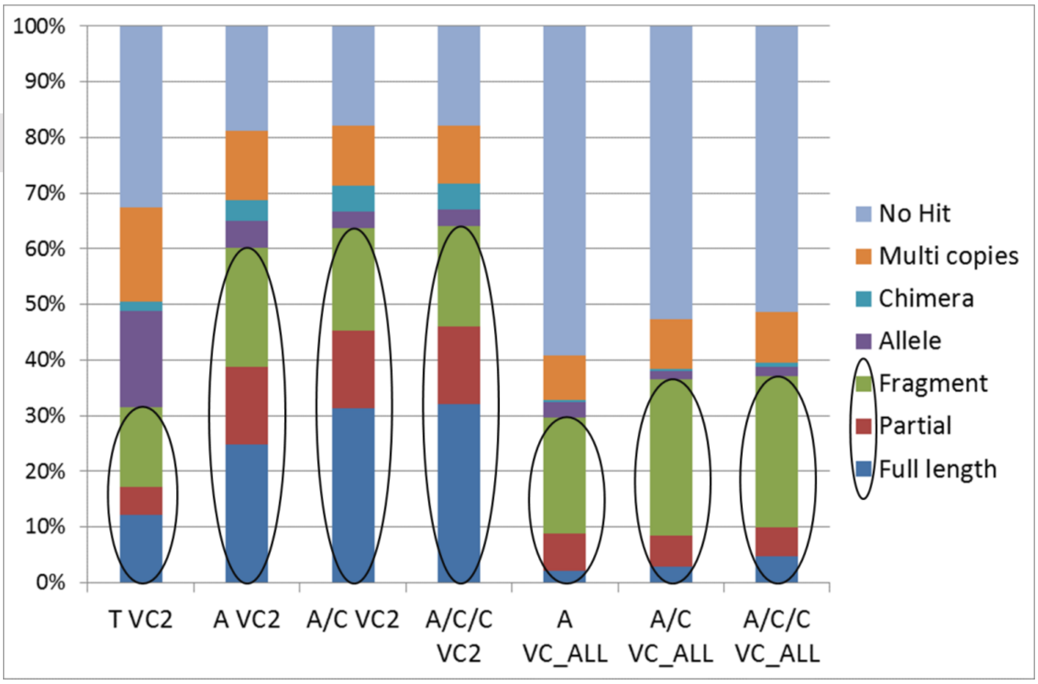


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Figure 5. Effect of RPKM filters on the ratio of non-problematic predicted contigs. The transcriptome assembly using Abyss with one step of Cap3 (A/C) and reads from the EC4 *Sorghum bicolor* individual (A/C EC4) maintained the best ratio of non-problematic predicted contigs (% of contigs from the categories Full length+Partial+Fragment) using different filters on RPKM : 0,1,2,3,4 (Reads per Kilobase per Million Reads).
T, Trinity; A, Abyss; C, Cap3; EC4, one individual *Sorghum bicolor* EC4; EC_ALL, 10 individuals *Sorghum bicolor*.

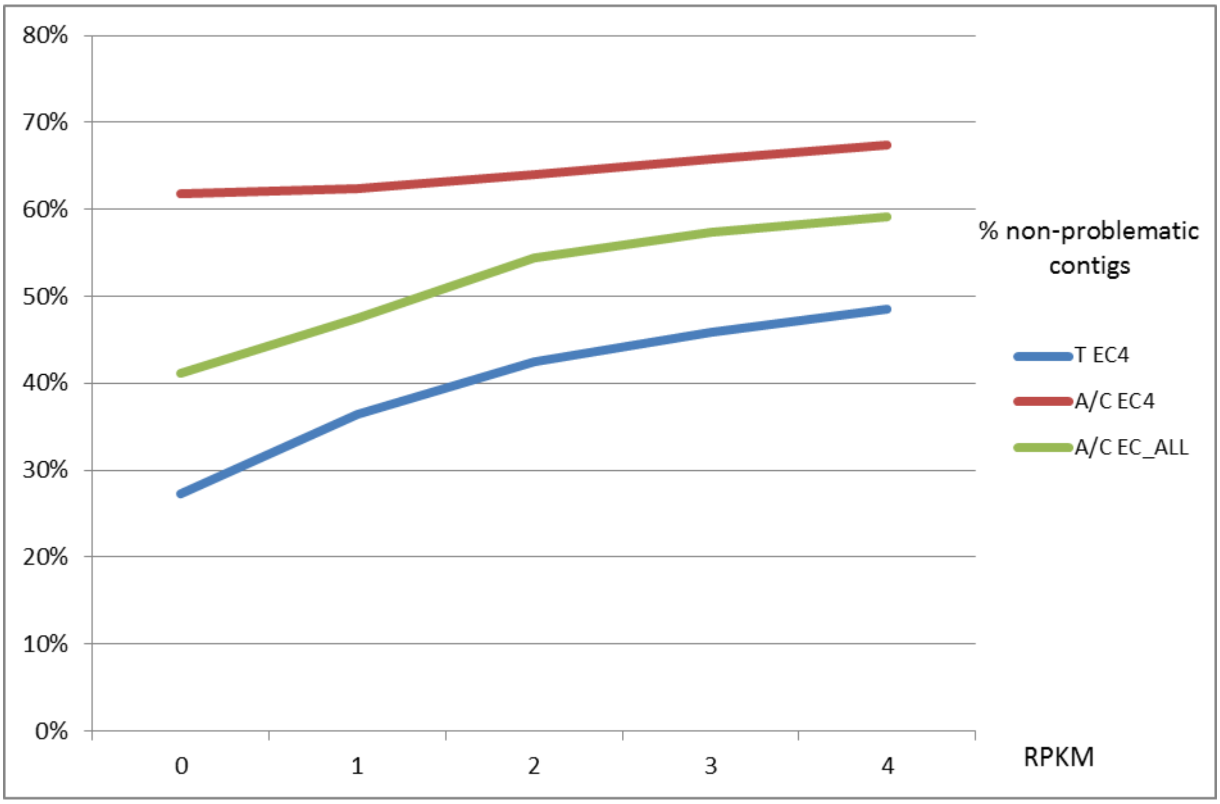

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Figure 6. Number of Best Blast Mutual Hits of our eggplant assembly with transcripts predicted from the draft genome SME_r2.5.1 (Kazusa), with a previously published transcriptome assembly from RNA-Seq (YanXu & al), and with a unigene set (Vegmark). The Venn diagram displays overlaps between the three data sets. Charts showing the list size (number of BBMH) and intersection size repartition are located underneath the diagram.

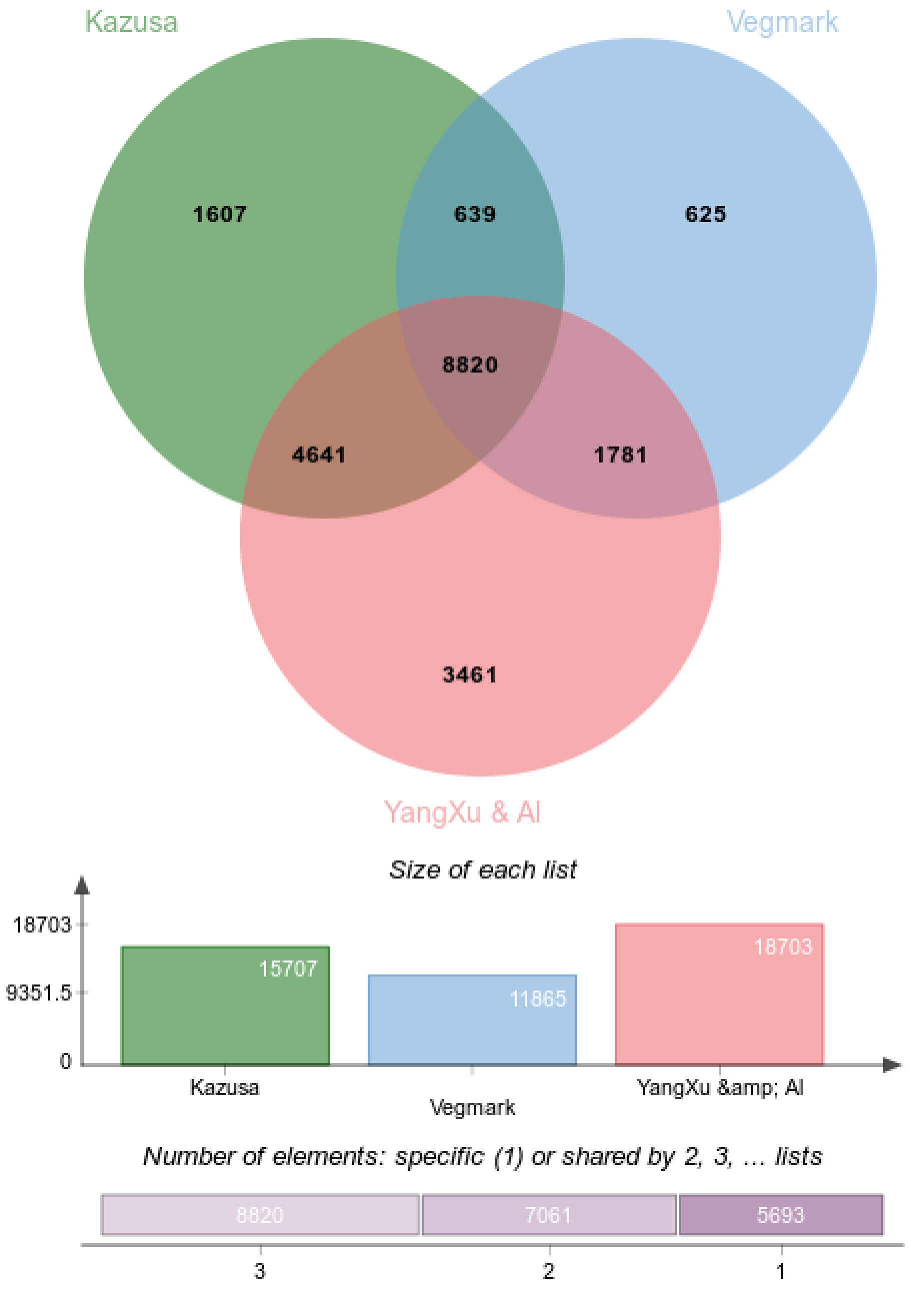

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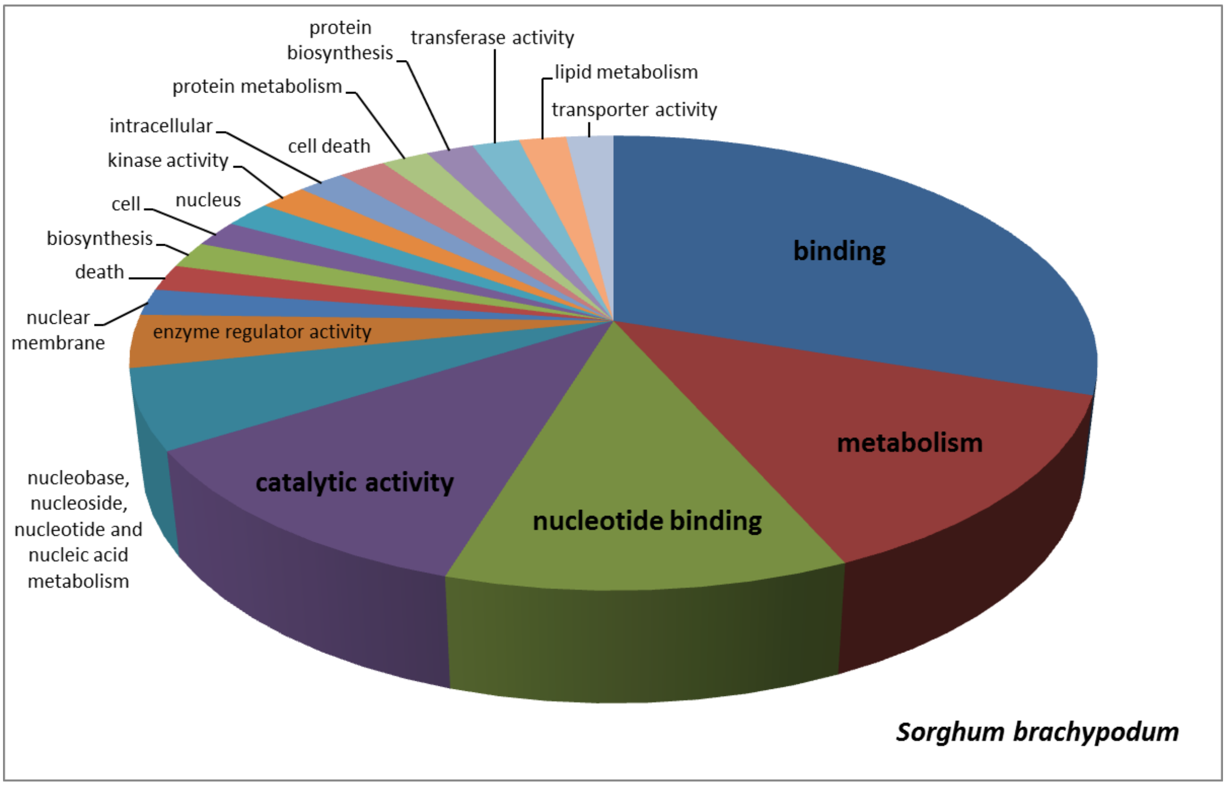
Figure 7. Main GO categories of the over-represented GO terms for *Sorghum brachypodum* contigs compared to other transcriptomes of the Poaceae family. The reference transcriptomes used for the Poaceae family were *Triticum monococcum, Eremopyrum bonaepartis, Taeniatherum caput-medusae, Pennisetum glaucum, Pennisetum polystachion, Pennisetum alopecuroides, Digitaria exilis,* and *Sorghum bicolor.*



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