The Pepper Genome

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Introduction to genomics (485) Spring 2019/2020



Outline

- Genome papers
- Fun facts
- General information about the genome
- Sequencing strategy
- Sequencing method
- Assembly
- Genome outcome
- Questions



Genome Papers

ARTICLES

genetics OPEN

Genome sequence of the hot pepper provides insights into the evolution of pungency in *Capsicum* species

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Whole-genome sequencing of cultivated and wild peppers provides insights into *Capsicum* domestication and specialization

Cheng Qin^{a,b,c,1}, Changshui Yu^{b,1}, Yaou Shen^{a,1}, Xiaodong Fang^{d,e,1}, Lang Chen^{b,1}, Jiumeng Min^{d,1}, Jiaowen Cheng^c, Shancen Zhao^d, Meng Xu^d, Yong Luo^b, Yulan Yang^d, Zhiming Wu^f, Likai Mao^d, Haiyang Wu^d, Changying Ling-Hu^b, Huangkai Zhou^d, Haijian Lin^a, Sandra González-Morales^g, Diana L. Trejo-Saavedra^h, Hao Tian^b, Xin Tang^c, Maojun Zhaoⁱ, Zhiyong Huang^d, Anwei Zhou^b, Xiaoming Yao^d, Junjie Cui^c, Wenqi Li^d, Zhe Chen^a, Yongqiang Feng^b, Yongchao Niu^d, Shimin Bi^b, Xiuwei Yang^b, Weipeng Li^c, Huimin Cai^d, Xirong Luo^b, Salvador Montes-Hernández^j, Marco A. Leyva-González^g, Zhiqiang Xiong^d, Xiujing He^a, Lijun Bai^d, Shu Tan^c, Xiangqun Tang^b, Dan Liu^d, Jinwen Liu^d, Shangxing Zhang^b, Maoshan Chen^d, Lu Zhang^{d,k}, Li Zhang^c, Yinchao Zhang^a, Weiqin Liao^b, Yan Zhang^d, Min Wang^b, Xiaodan Lv^d, Bo Wen^d, Hongjun Liu^a, Hemi Luan^d, Yonggang Zhang^b, Shuang Yang^d, Xiaodian Wang^b, Jiaohui Xu^d, Xueqin Li^b, Shuaicheng Li^k, Junyi Wang^d, Alain Palloix^l, Paul W. Bosland^m, Yingrui Li^d, Anders Krogh^e, Rafael F. Rivera-Bustamante^h, Luis Herrera-Estrella^{g,2}, Ye Yin^{d,2}, Jiping Yu^{b,2}, Kailin Hu^{c,2}, and Zhiming Zhang^{a,2}

Fun Facts

- Genus name 'Capsicum' comes from the Greek kapto means 'to bite'.
- The heat sensation (pungency) is due to capsinoids produced by pepper.
- Birds don't have the receptor for capsinoid, so they don't feel the hot sensation as mammals.
- Pepper is the richest in vitamin C content, even than lemon.

 1. VELLOW RELL PEPPERS 341.3 mg for a large pepper



General information about the genome

- Belong to Solanacea family, with tomato and potato.
- 12 pairs of chromosomes (diploid).
- Linear, dsDNA.
- Genome size: 3.48 Gb for Capsicum annuum specie



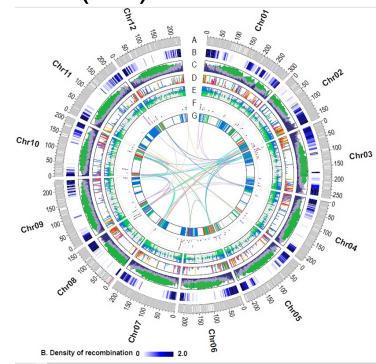


General information about the genome

Around 35,000 protein coding genes → 3,143
genes of them are specific for pepper, and over
31% of the protein coding genes are housekeeping,
located almost near the telomeres.

The genome transposable elements (TE) ≈ 2.7Gb

larger than human genome!



General information about the genome

- The genomes sequenced are for:
 - ☐ Capsicum annuum L.



☐ C.annuum Zunla-1

☐ C.annuum Chiltepin

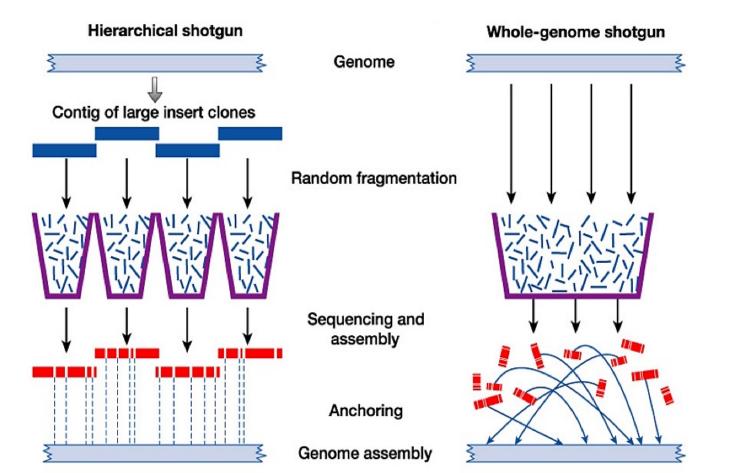




Sequencing Strategy

Hierarical- BAC clones liberaries (for validation)

Whole Genome Shotgun (WGS)



Sequencing Method

Sanger sequencing Genomic DNA Illumina NGS sequencing Sanger sequencing **DNA** denaturation Apply heat to convert dsDNA into ssDNA 11111111111 Library<u>......</u> **DNA** denaturation Genomic DNA is sonicated preparation into 200-700 bp long шиши 11111111111111111111111111 fragments Adapter ligation шинини Small sequences of DNA **DNA** amplification called adapters are ligated to the DNA fragments Cluster Chain reactions amplification DNA fragments are added to Four reactions are set, each containing: a flow cell. Adapters bind to - template DNA + primer their complementary oligos, and DNA fragments attach. - DNA polymerase free nucleotides - marked nucleotides (a) A reverse strand of the DNA fragment is synthesized, (b) the dsDNA molecule is denatured, and (c) the original DNA template is washed away. The strands are amplified through bridge amplification, where (a) the strand folds over and its free end binds to the nearest oligo; (b) the complementary DNA strand is synthesized, forming a double-stranded forming two ssDNA strands. This process is repeated over and over, and occurs simultaneously for millions of clusters, resulting in clonal amplification of the original fragments. After bridge amplification, (a) Gel eletrophoresis the ds molecules are denatured and (b) all R strands are cleaved and washed off, (c) leaving only the F strands.

sequence

Illumina (current) sequencing

After each round of amplification, lasers are passed over the flow cell to activate the fluorescent label on the nucleotide bases. This fluorescence

is detected by a camera and recorded on a computer. Each of the bases gives off a different colour.

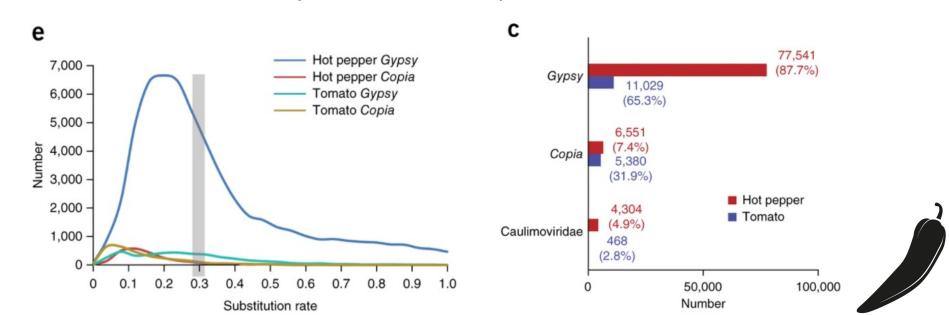


Assembly

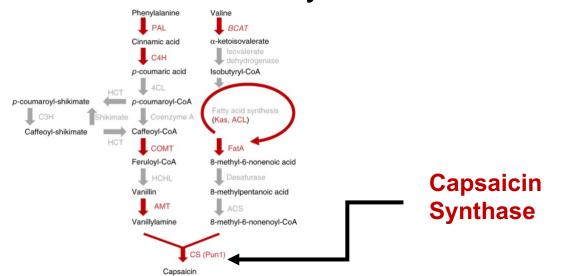
	Assembly method	Genome size	Coverage	GC%	N50 contig	N50 scaffold
Capsicum annuum (hot)	SOAPdenovo & SSPACE	3.48 Gb	186.6 x	35.03 %	30 kb	2.47 Mb
C. annuum Zunla-1 (cultivated)	SOAPdenovo	3.26 Gb	99 x	34.9 %	55.4 kb	1.23 Mb
C. annuum Chiltepin (wild)	SOAPdenovo	3.07 Gb	67 x	35 %	52.2 kb	0.445 Mb

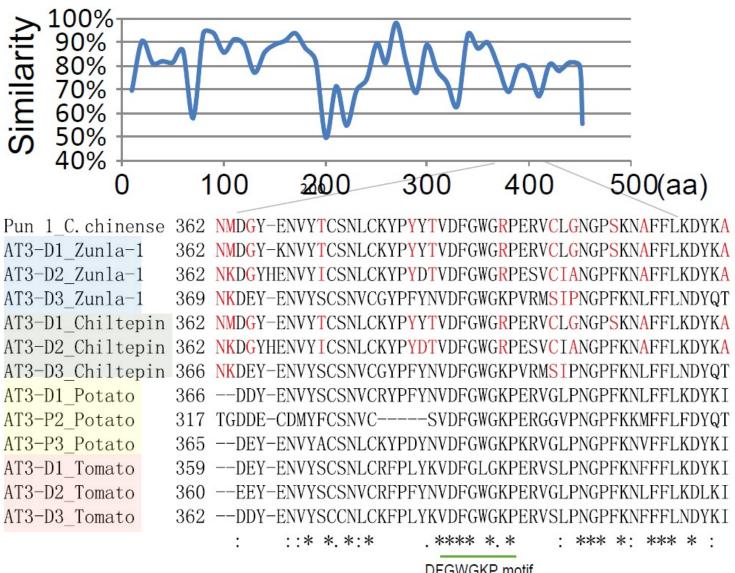


- Genome size much larger than its family members due to rapid amplification of retrotransposable elements.
- 81% of the genome are transposable elements (TE)!
- LTR is the most, 70% parallel the maize genome, 75%.
- Most of the LTRs are Gypsy that are the main cause of genome expansion.
- Gypsy is loaded with reverse transcriptase domains (12 fold than other types of LTRs).



- Whole genome triplication happen > most genes lost one or two copies > few remaining triplicated genes.
- Example: AT3 (Pun1) gene code for capsaicin synthase (CS) that have a conserved motif.
- The gene have two alleles:
 - AT3-D1 > non-pungent, due to deletions in the promoter and first exon (pseudogene)
 - AT3-D2 > Pungent, code for CS that contribute in the capsaicinoid synthesis pathway.





DFGWGKP motif

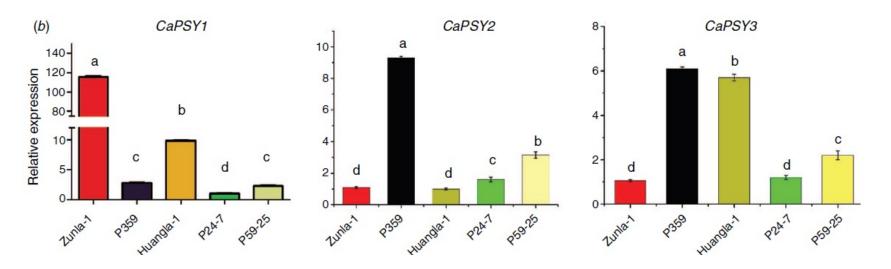
- You may ask, why presenting a lot of red in the although you see variation in pepper colors in you Salad?
- This is because of the level of CaPSY1 gene expression level that thought to be associated with the red and yellow colors of the pepper specie.

CSIRO PUBLISHING

Functional Plant Biology https://doi.org/10.1071/FP19287

CaPSY1 gene plays likely the key role in carotenoid metabolism of pepper (Capsicum annuum) at ripening





Questions

- Order the assembly quality for the three strains starting from the best. Justify the answer.
- What is the reason behind variation in the pungency (hot sensation) of different pepper species?

