Alfalfa Genome Sequencing
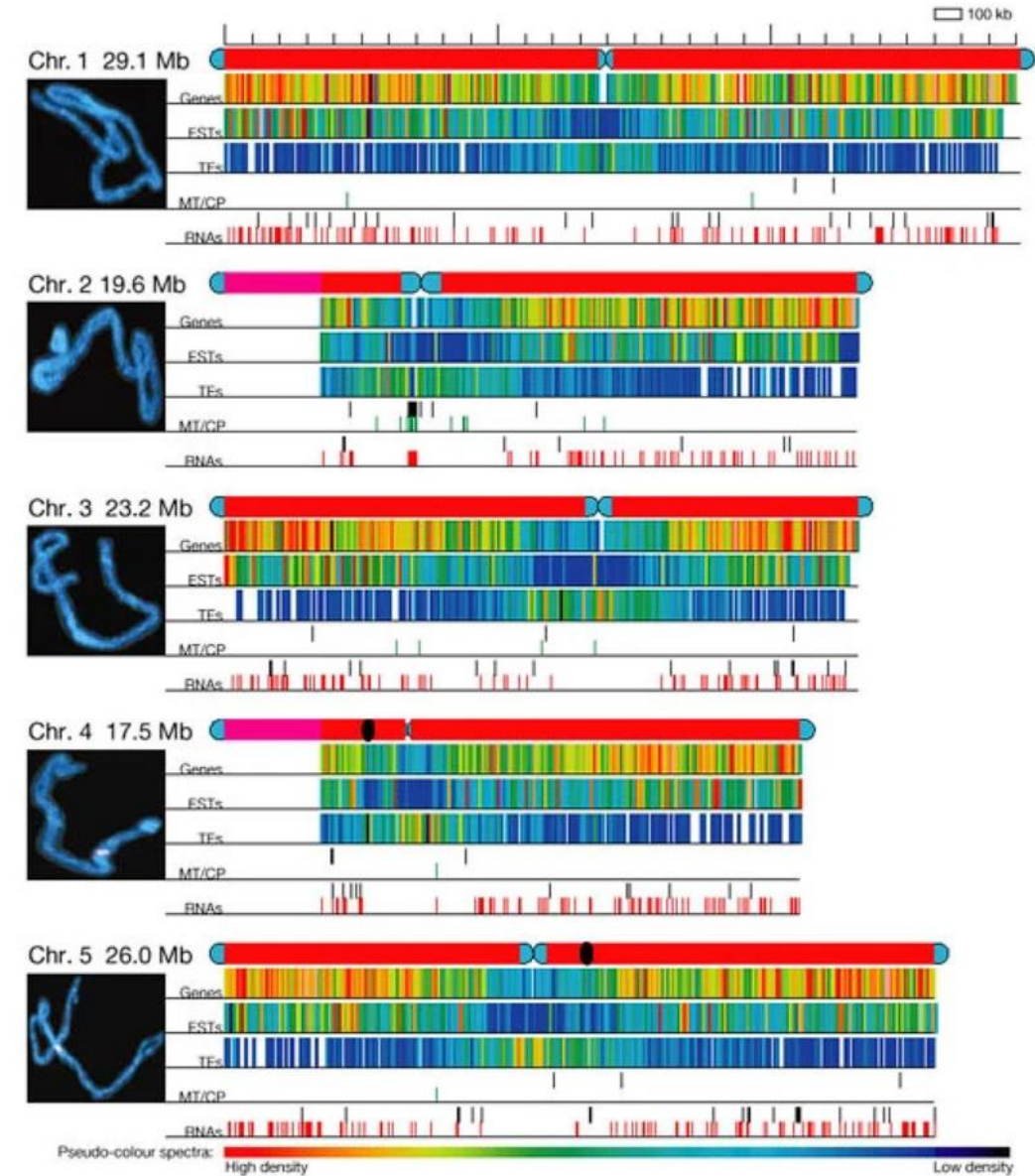
Tao Wang

China Agricultural University

2022.11.15
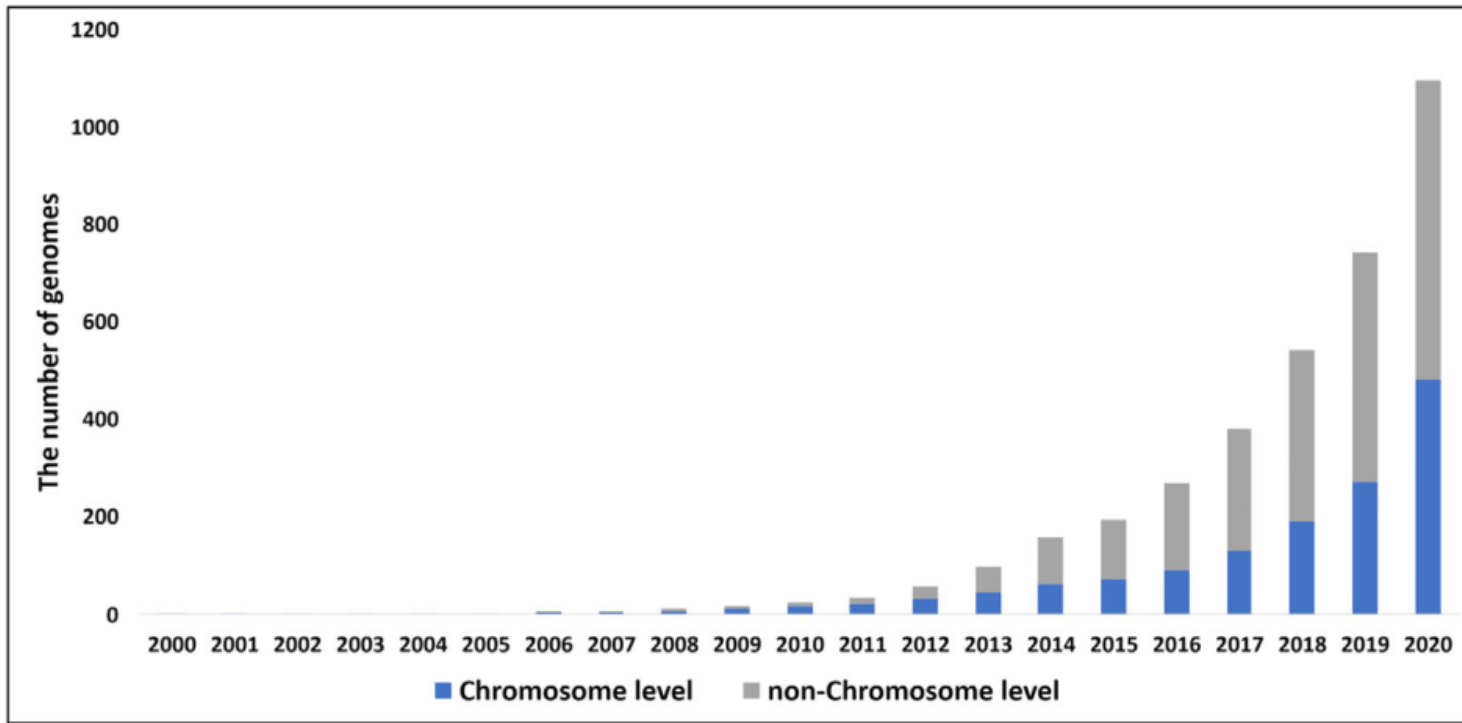
Plant Genome Program

● **The publication of the first plant reference genome - the whole genome sequence of *Arabidopsis thaliana* in December 2000 marked the beginning of the plant genome era.**
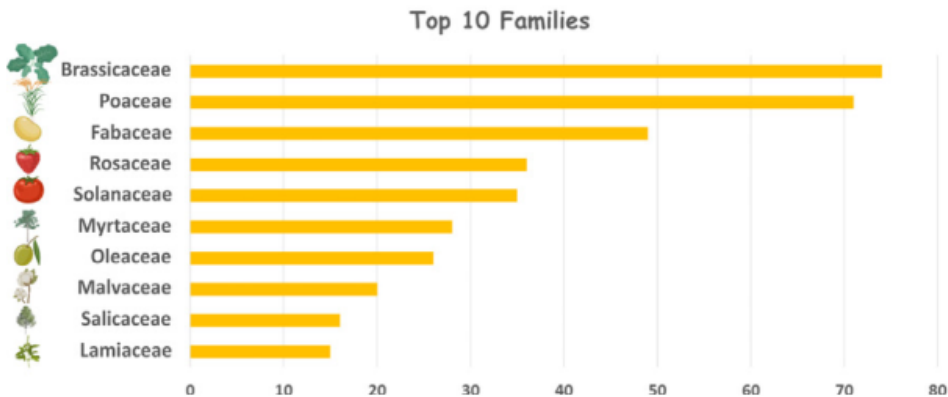
Arabidopsis Genome Initiative, *Nature*, 2000
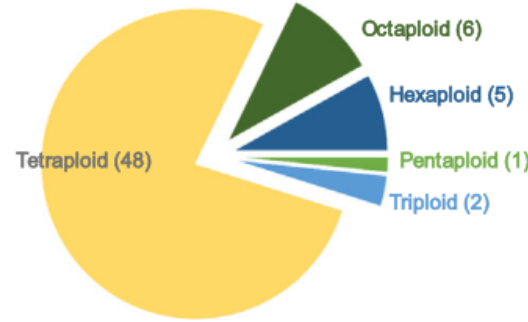
(A)

(B) Top 10 Families

(C) Trends in Plant Science

- **By the end of 2020, 1031 genomes of 788 different plant species (including subspecies) have been sequenced and published, and the number continues to grow exponentially.**
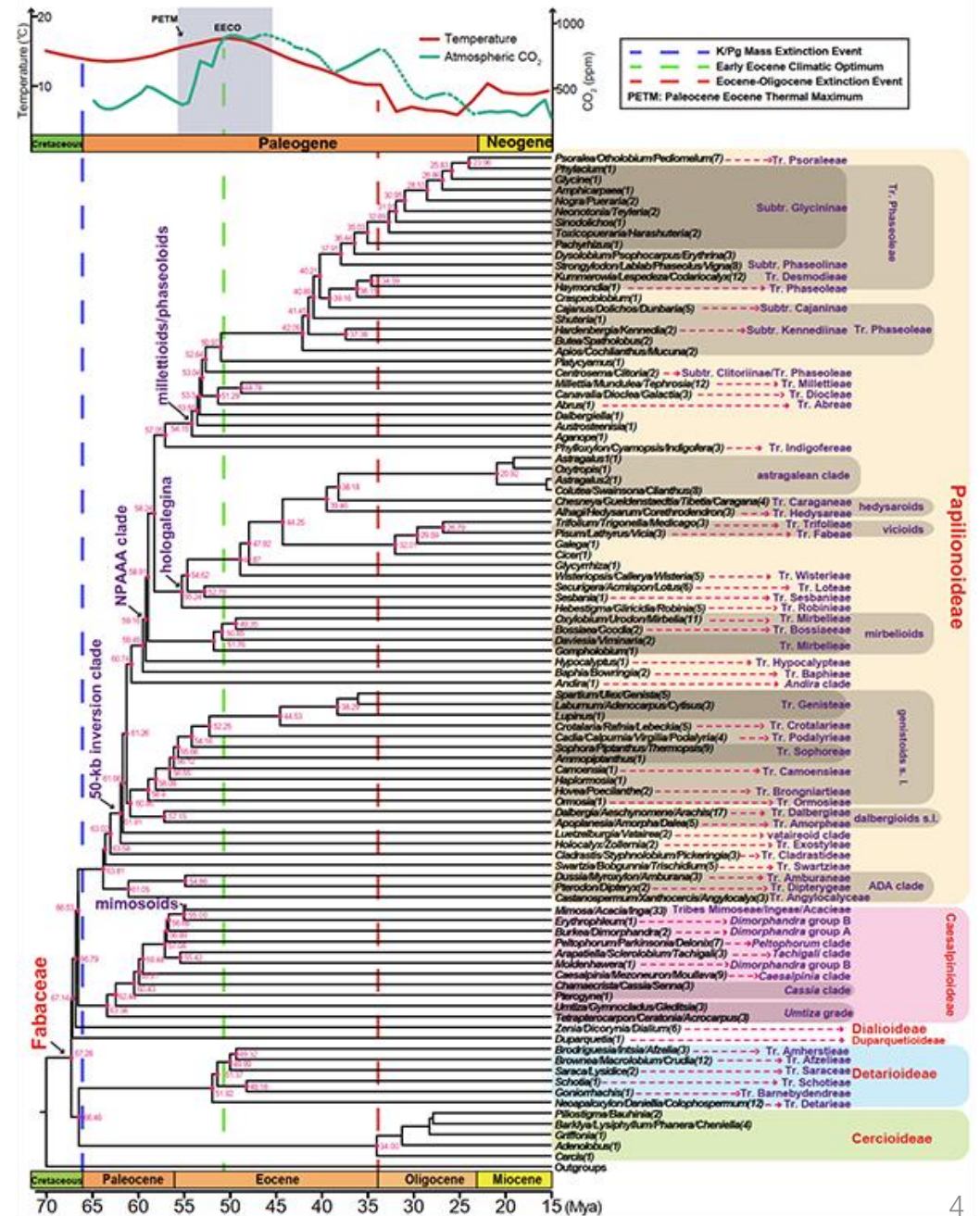
- **The most sequenced species were *Brassicaceae*, *Poaceae*, and *Fabaceae*.**

- **Tetraploids are the most sequenced in polyploid genomes sequenced in angiosperms.**

Sun et al., *Trends Plant Sci.*, 2021

Zhao et al., *Mol. Plant*, 2021

Medicago sativa

- High protein, high cellulose, "the Queen of Forages".

- Autotetraploid, cross-pollinated, high genomic heterozygosity.

- Capable of nitrogen fixation by nodules.

- Capable of genetic transformation.

Medicago truncatula

- An ideal model plant for the legume family.

- Annual, self-pollinated, diploid.

- Capable of nitrogen fixation by nodules.

- Capable of genetic transformation.

Medicago genome

- **The sequencing program of the *Medicago* genome started in 2003.**

- **The *Medicago* genome (Mt3.5) was finally published in Nature in 2011.**

- **About 250 Mb of sequences was assembled, representing most of the chromatin, but more than 100 Mb of sequences was not assembled.**

M. truncatula **Jemalong A17**

The *Medicago* genome provides insight into the evolution of rhizobial symbioses

Nevin D. Young ✉, Frédéric Debellé, Giles E. D. Oldroyd, Rene Geurts, Steven B. Cannon, Michael K. Udvardi, Vagner A. Benedito, Klaus F. X. Mayer, Jérôme Gouzy, Heiko Schoof, Yves Van de Peer, Sebastian Proost, Douglas R. Cook, Blake C. Meyers, Manuel Spannagl, Foo Cheung, Stéphane De Mita, Vivek Krishnakumar, Heidrun Gundlach, Shiguo Zhou, Joann Mudge, Arvind K. Bharti, Jeremy D. Murray, Marina A. Naoumkina, … Bruce A. Roe ➕ Show authors
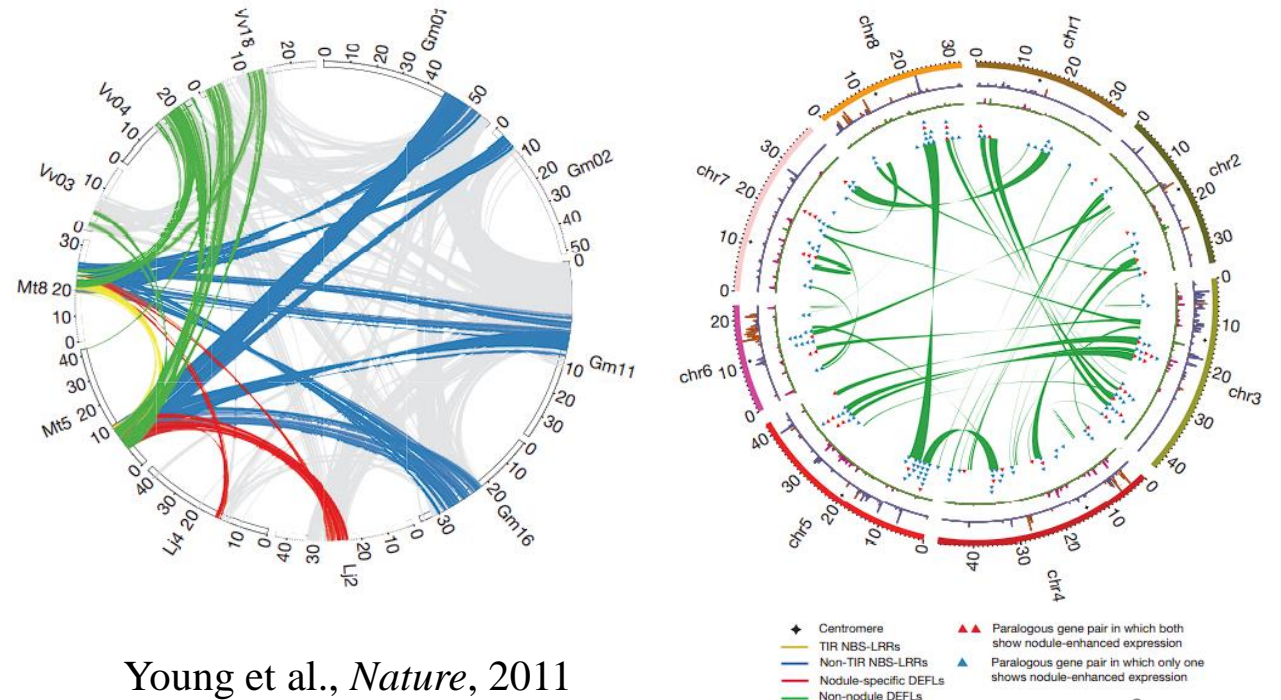
Young et al., *Nature*, 2011

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- **The *M. truncatula* genome underwent two updates, and the most complete reference genome (Mt 5.0) was obtained so far in 2018.**
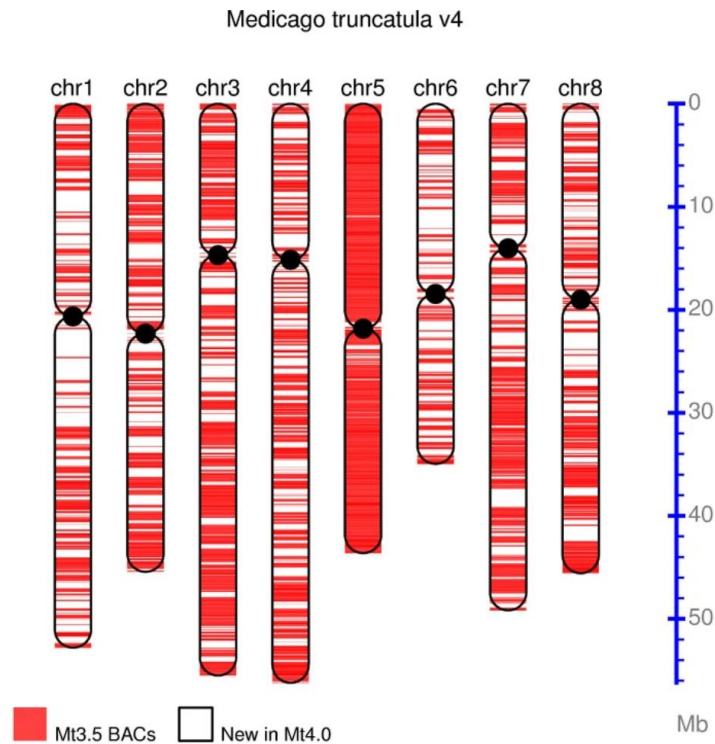
An improved genome release (version Mt4.0) for the model legume Medicago truncatula

Haibao Tang, Vivek Krishnakumar, Shelby Bidwell, Benjamin Rosen, Agnes Chan, Shiguo Zhou, Laurent Gentzbittel, Kevin L Childs, Mark Yandell, Heidrun Gundlach, Klaus FX Mayer, David C Schwartz & Christopher D Town ✉

Whole-genome landscape of *Medicago truncatula* symbiotic genes

Yann Pecrix, S. Evan Staton, Erika Sallet, Christine Lelandais-Brière, Sandra Moreau, Sébastien Carrère, Thomas Blein, Marie-Françoise Jardinaud, David Latrasse, Mohamed Zouine, Margot Zahm, Jonathan Kreplak, Baptiste Mayjonade, Carine Satgé, Magali Perez, Stéphane Cauet, William Marande, Céline Chantry-Darmon, Céline Lopez-Roques, Olivier Bouchez, Aurélie Bérard, Frédéric Debellé, Stéphane Muños, Abdelhafid Bendahmane, ... Pascal Gamas ✉ [+ Show authors]

Tang et al., *BMC Genomic*, 2014
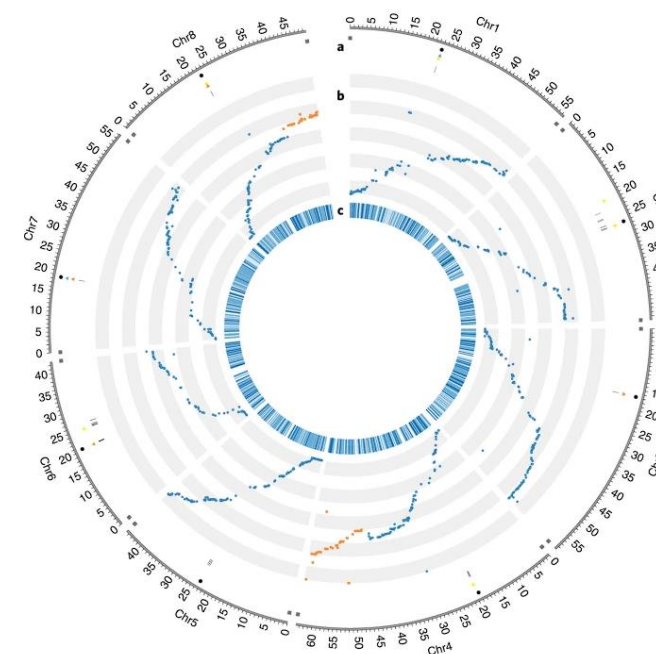
Pecrix et al., *Nat. Plant*, 2018

Medicago genome – R108

Strategies for optimizing BioNano and Dovetail explored through a second reference quality assembly for the legume model, *Medicago truncatula*

Karen M. Moll, Peng Zhou, Thiruvarangan Ramaraj, Diego Fajardo, Nicholas P. Devitt, Michael J. Sadowsky, Robert M. Stupar, Peter Tiffin, Jason R. Miller, Nevin D. Young, Kevin A. T. Silverstein & Joann Mudge ✉
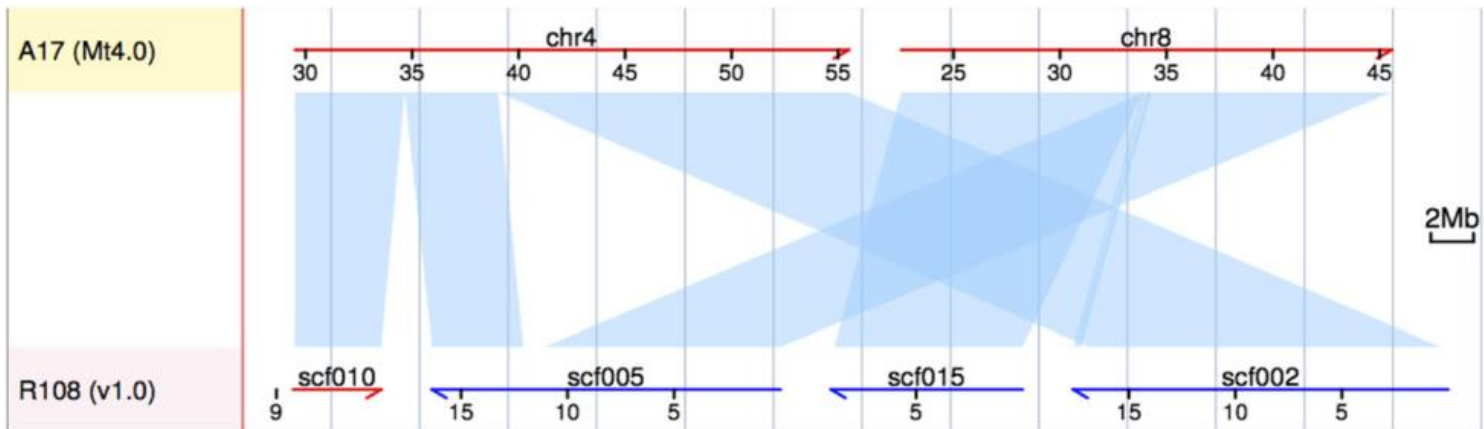
Compared with A17, R108 has higher transformation efficiency and is more advantageous for genetic studies.


Moll et al., *BMC Genomic*, 2017

Synteny alignment of partial chromosomes 4 and 8 between A17 and R108 confirms the rearrangement of the long arms of the chromosomes.

Alfalfa genome – Zhongmu No.1

The Chromosome-Level Genome Sequence of the Autotetraploid Alfalfa and Resequencing of Core Germplasms Provide Genomic Resources for Alfalfa Research

Chen Shen[1,4], Huilong Du[2,3,4], Zhuo Chen[2,3,4], Hongwei Lu[2,3,4], Fugui Zhu[1], Hong Chen[1], Xiangzhao Meng[1], Qianwen Liu[1], Peng Liu[1], Lihua Zheng[1], Xiuxiu Li[2,3], Jiangli Dong[1,*], Chengzhi Liang[2,3,*] and Tao Wang[1,*]
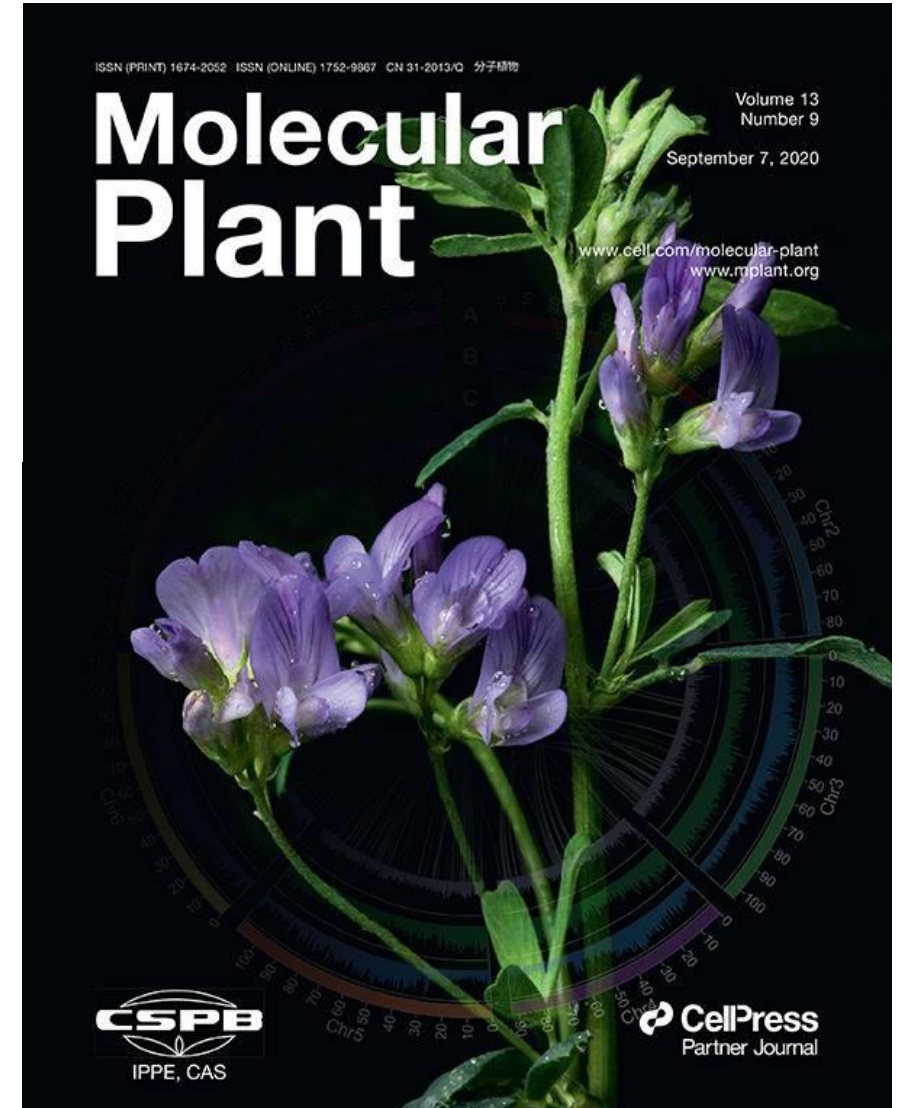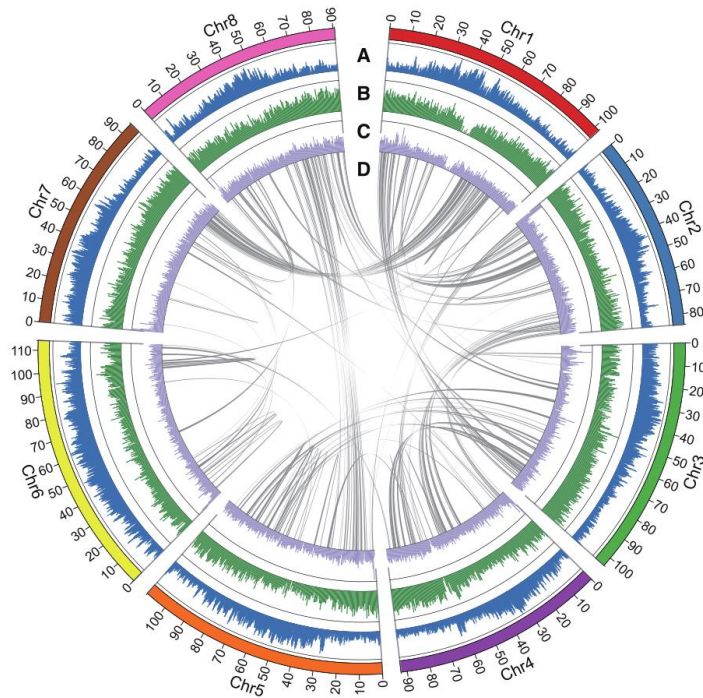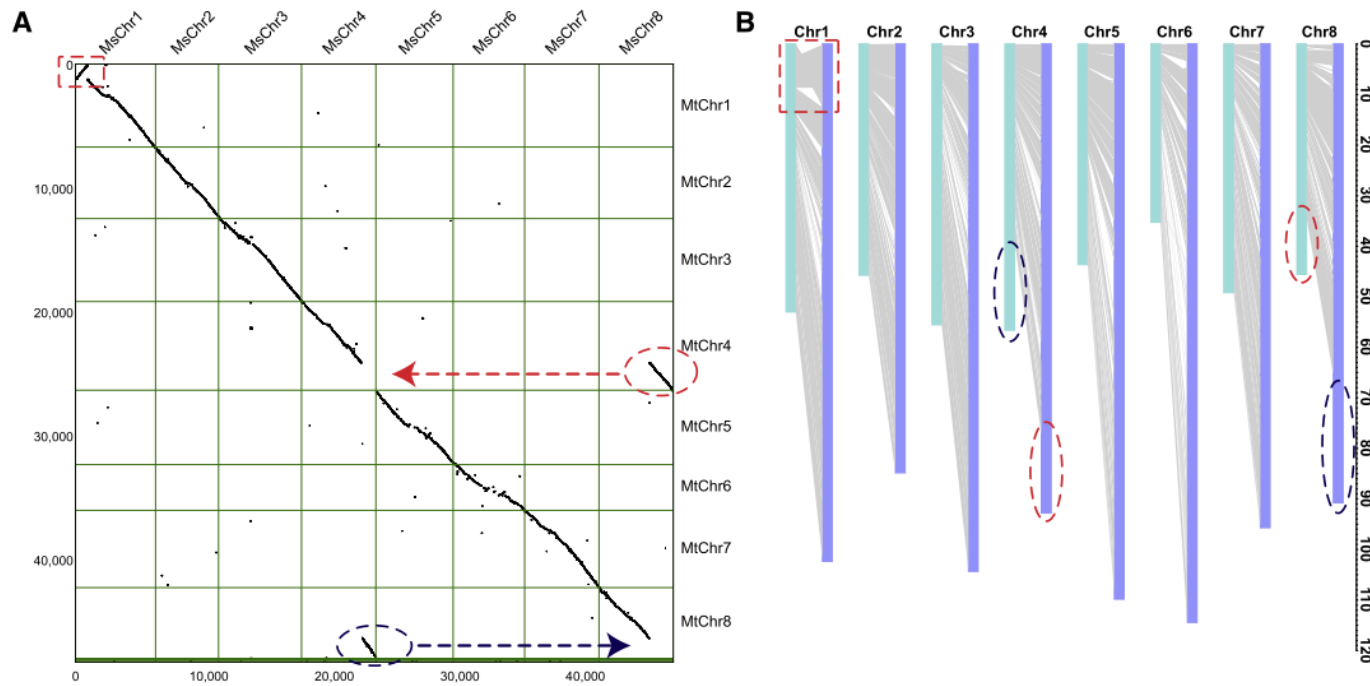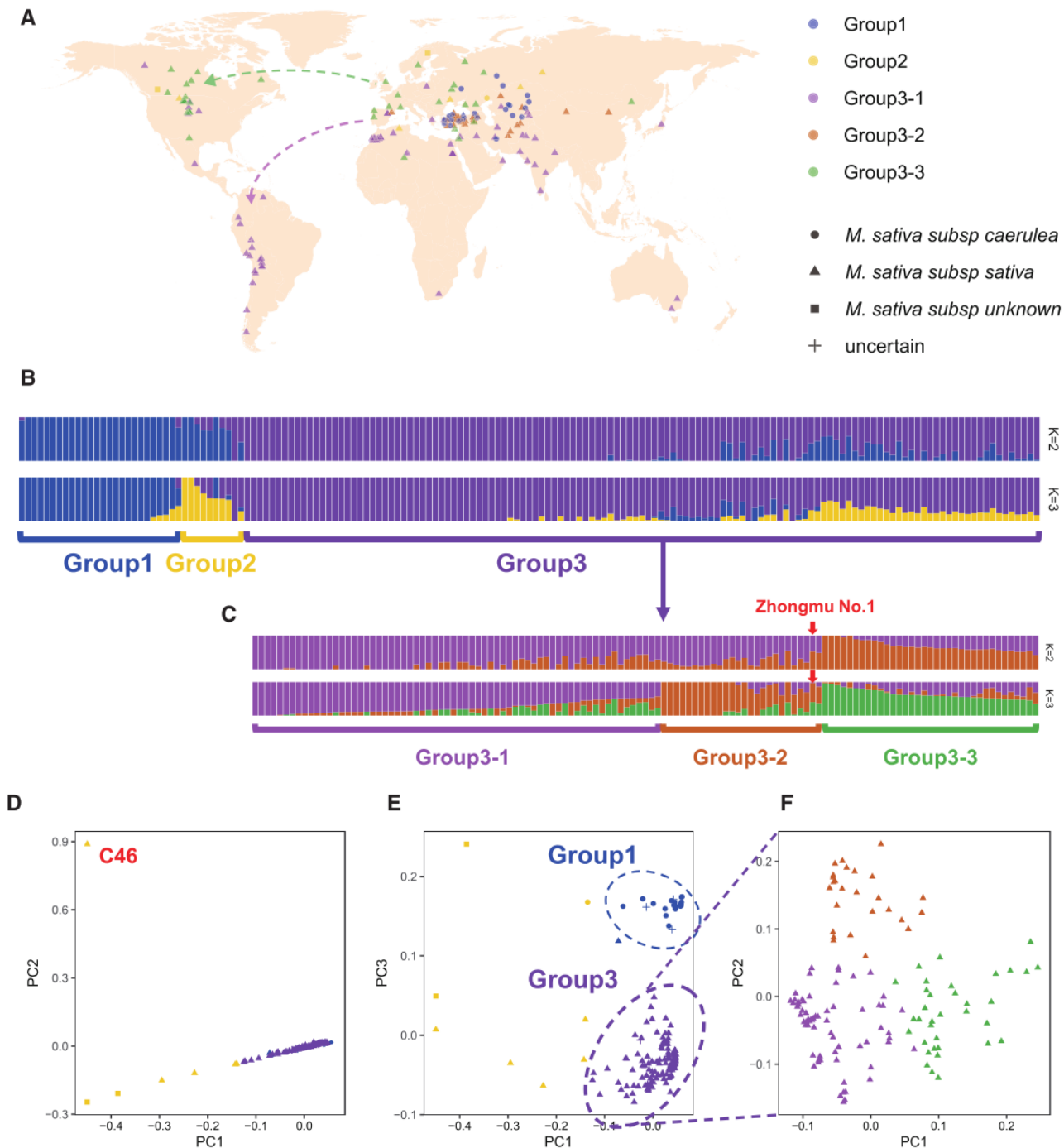
A. Comparative analysis of the Zhongmu No. 1 genome and the *M. truncatula* genome showed that they were highly collinear.

B. There was a large translocation (~20 Mb) between chromosomes 4 and 8 and an inversion in chromosome 1.
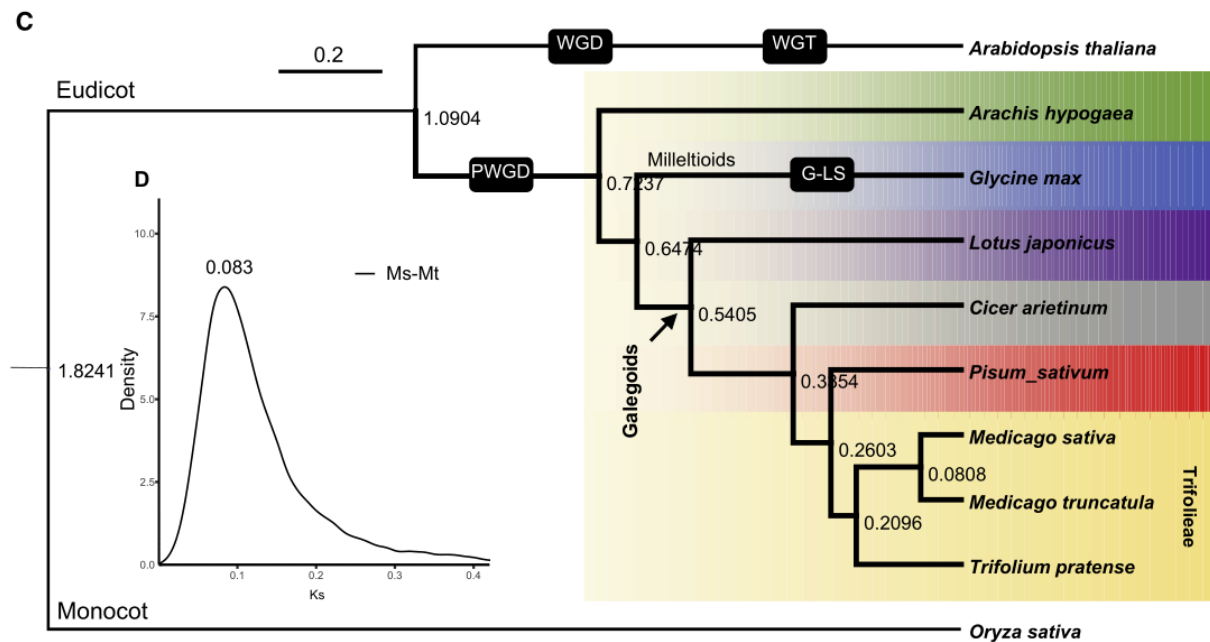
C. Phylogenetic tree showed that alfalfa is most closely related to *M. truncatula*.

Shen et al., *Mol. Plant*, 2020

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- **Group 1 was dominated by ssp. *caerulea*; group 2 contained two unknown diploid samples and some admixed tetraploid samples; group 3 mainly contained tetraploid alfalfa.**
- **Group 3 could be further divided into three subgroups that were highly correlated with subspecies geographic distribution.**
- **Cultivated alfalfa is inferred to have originated from an ancestral group in Europe.**

Shen et al., *Mol. Plant*, 2020
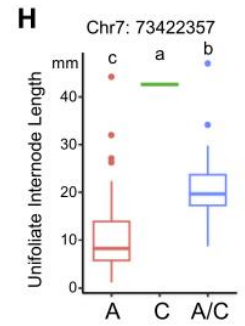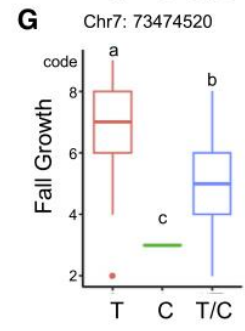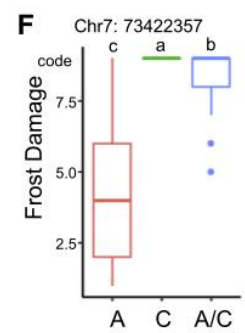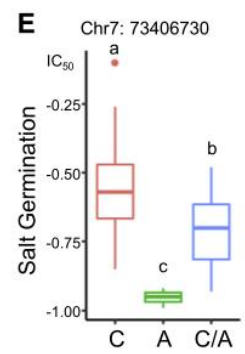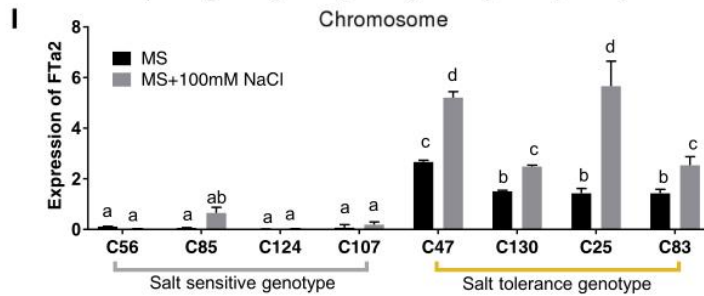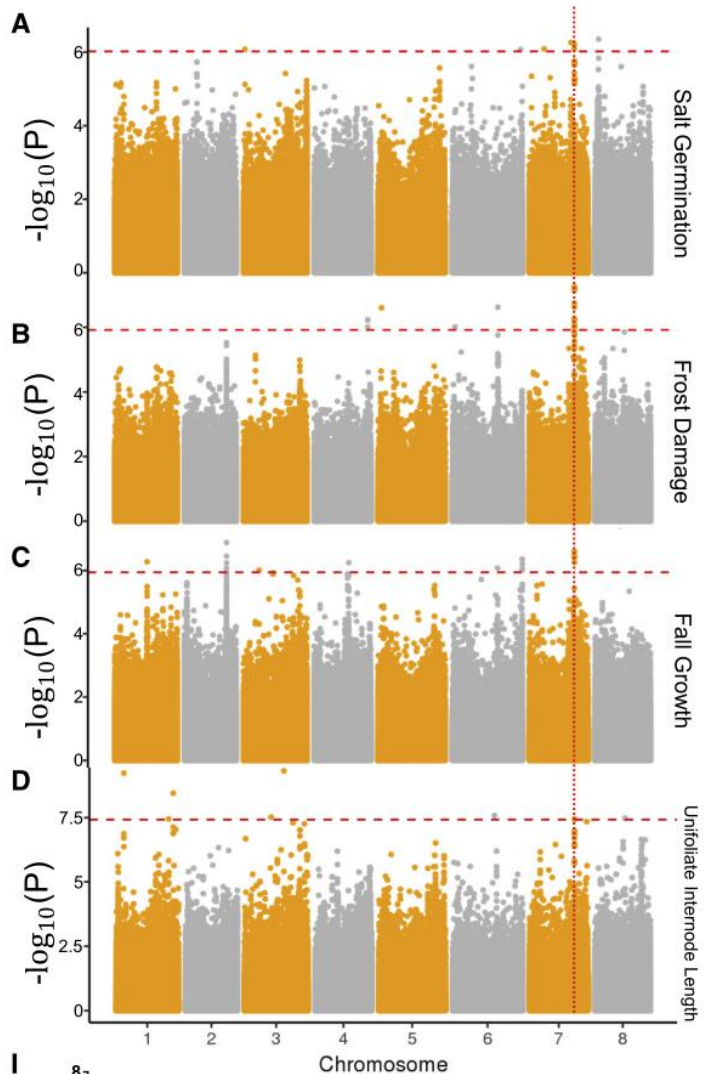
- We performed GWAS on the 137 global core germplasms using whole-genome SNP data and phenotypic data from the U.S. National Plant Germplasm System.

- Using 2,463,637 biallelic SNPs based on the Zhongmu No. 1 genome, we identified more than 100 candidate regions associated with more than 30 agronomic traits, including disease resistance, insect resistance, growth, morphology, productivity, and stress response.

- These results identified valuable genomic markers and candidate genes that can be used in future breeding and basic research efforts.

Shen et al., *Mol. Plant*, 2020

Alfalfa genome – XinJiangDaYe

Allele-aware chromosome-level genome assembly and efficient transgene-free genome editing for the autotetraploid cultivated alfalfa

Haitao Chen, Yan Zeng, Yongzhi Yang, Lingli Huang, Bolin Tang, He Zhang, Fei Hao, Wei Liu, Youhan Li, Yanbin Liu, Xiaoshuang Zhang, Ru Zhang, Yesheng Zhang, Yongxin Li, Kun Wang, Hua He, Zhongkai Wang, Guangyi Fan, Hui Yang, Aike Bao, Zhanhuan Shang, Jianghua Chen ✉, Wen Wang ✉ & Qiang Qiu ✉

- **Genome assembly of XinJiangDaYe**
- **CRISPR/Cas9 system**

Chen et al., *Nat. Commun*, 2020

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Alfalfa genome – Zhongmu No.4

Genome Assembly of Alfalfa Cultivar
Zhongmu-4 and Identification of SNPs
Associated with Agronomic Traits

Ruicai Long [1, #], Fan Zhang [1, 2, #], Zhiwu Zhang [2], Mingna Li [1], Lin Chen [1], Xue Wang [1], Wenwen Liu [1], Tiejun Zhang [3], Long-Xi Yu [4], Fei He [1], Xueqian Jiang [1], Xijiang Yang [1], Changfu Yang [1], Zhen Wang [1] ⚲ ✉, Junmei Kang [1] ⚲ ✉, Qingchuan Yang [1] ⚲ ✉
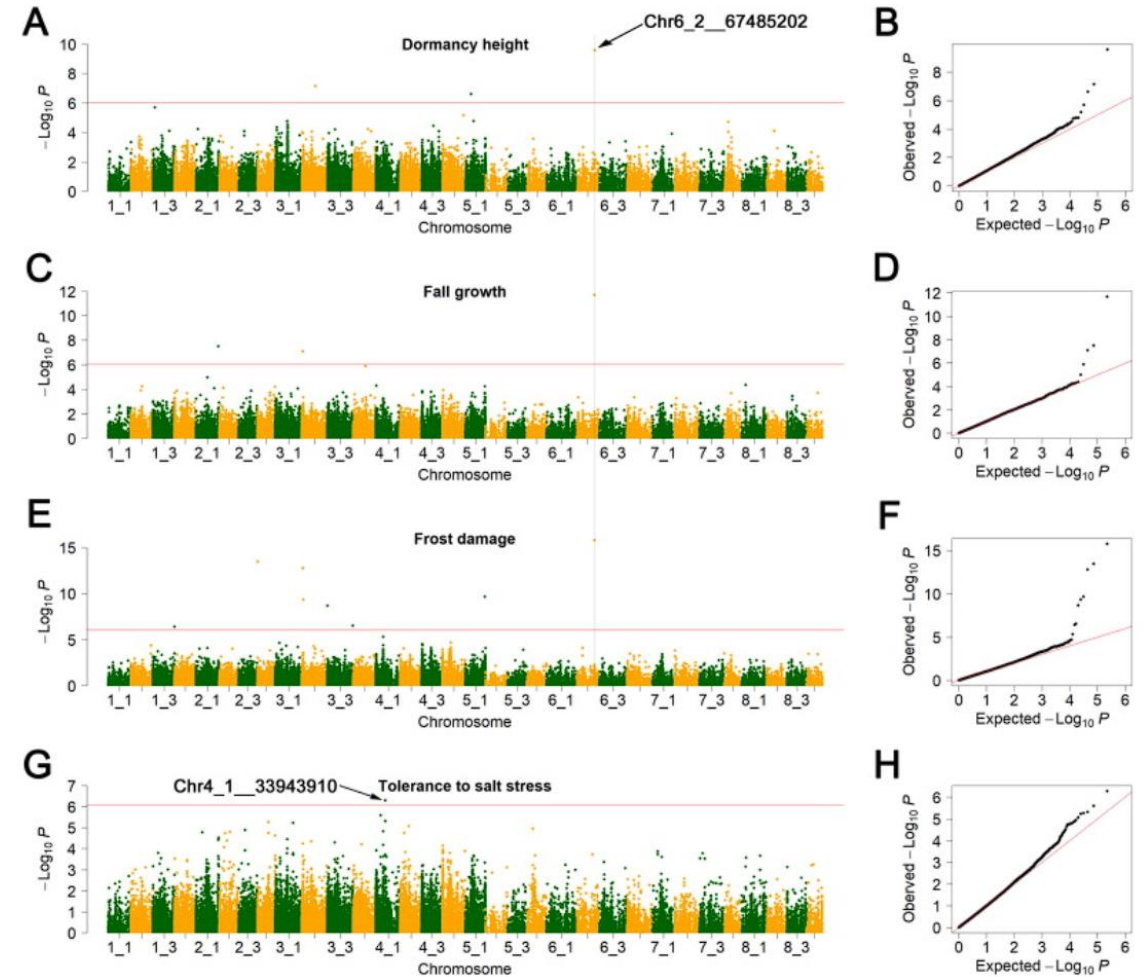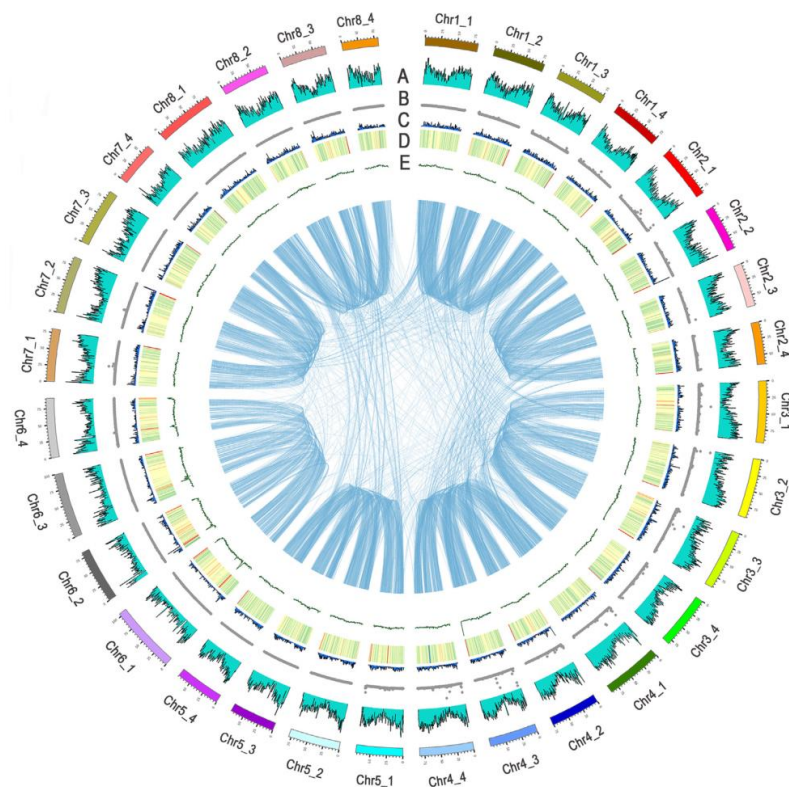
Long et al., *Genomics Proteomics Bioinformatics*, 2022

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Article | Open Access | Published: 01 December 2020

A chromosome-scale genome assembly of a diploid alfalfa, the progenitor of autotetraploid alfalfa

Ao Li, Ai Liu, Xin Du, Jin-Yuan Chen, Mou Yin, Hong-Yin Hu, Nawal Shrestha, Sheng-Dan Wu, Hai-Qing Wang, Quan-Wen Dou, Zhi-Peng Liu, Jian-Quan Liu, Yong-Zhi Yang ✉ & Guang-Peng Ren ✉

4655 Accesses | **10** Citations | **1** Altmetric | Metrics

Li et al., *Hort. Res.*, 2020

The genome of *Medicago polymorpha* provides insights into its edibility and nutritional value as a vegetable and forage legume

Jiawen Cui, Zhaogeng Lu, Tianyi Wang, Gang Chen, Salma Mostafa, Hailong Ren, Sian Liu, Chunxiang Fu, Li Wang, Yingfang Zhu, Jinkai Lu, Xiang Chen, Zhenwu Wei ✉ & Biao Jin ✉

3334 Accesses | **8** Citations | **1** Altmetric | Metrics

Cui et al., *Hort. Res.*, 2021

RESEARCH ARTICLE   **Open Access**

The genome of a wild *Medicago* species provides insights into the tolerant mechanisms of legume forage to environmental stress

Tianzuo Wang[1], Lifei Ren[1], Caihong Li[1], Di Zhang[1], Xiuxiu Zhang[1], Gang Zhou[2], Dan Gao[2], Rujin Chen[3], Yuhui Chen[3], Zhaolan Wang[4], Fengling Shi[5], Andrew D. Farmer[6], Yansu Li[7], Mengyan Zhou[2*], Nevin D. Young[8] and Wen-Hao Zhang[1,9*]

Wang et al., *BMC Bio.*, 2021
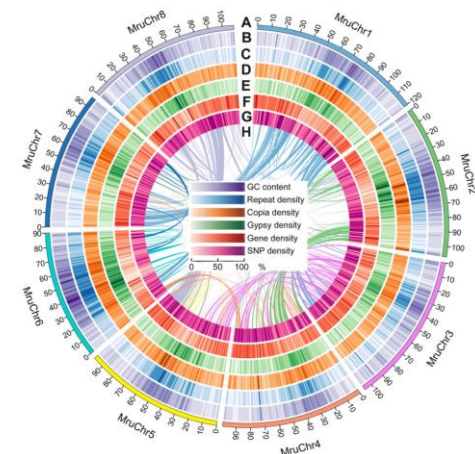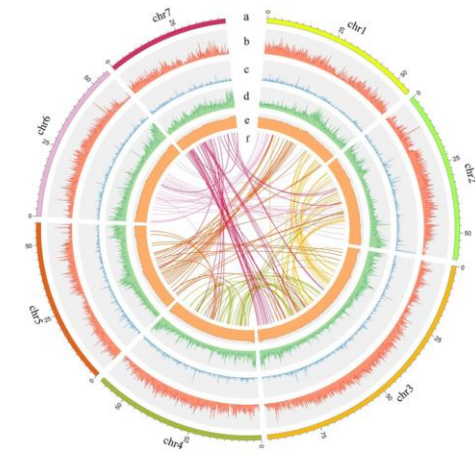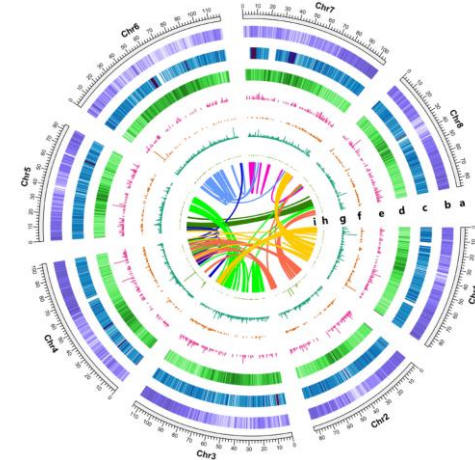
| Time	Article	Varieties	Size	Contig N50
2017	Strategies for optimizing BioNano and Dovetail explored through a second reference quality assembly for the legume model, Medicago truncatula	Medicago truncatula R108	400Mb	6Mb
2018	Whole-genome landscape of Medicago truncatula symbiotic genes	Medicago truncatula A17	430Mb	18Kb
2020	Allele-aware chromosome-level genome assembly and efficient transgene-free genome editing for the autotetraploid cultivated alfalfa	Alfalfa XinJiangDaYe	2.74Gb	459Kb
2020	The Chromosome-Level Genome Sequence of the Autotetraploid Alfalfa and Resequencing of Core Germplasms Provide Genomic Resources for Alfalfa Research	Alfalfa ZhongMu No.1	Total:3.2Gb High-quality:816Mb	3.9Mb
2020	A chromosome-scale genome assembly of a diploid alfalfa, the progenitor of autotetraploid alfalfa	diploid alfalfa	793Mb	3.86Mb
2021	The genome of a wild Medicago species provides insights into the tolerant mechanisms of legume forage to environmental stress	Medicago ruthenica	904Mb	613Kb
2021	The genome of Medicago polymorpha provides insights into its edibility and nutritional value as avegetable and forage legume	Medicago polymorpha	460Mb	11Mb
2022	Genome Assembly of Alfalfa Cultivar Zhongmu-4 and Identification of SNPs Associated with Agronomic Traits	Alfalfa ZhongMu No.4	2.74Gb	2.06Mb

Prospect – Gapless genome assembly

Research Report

Gapless *indica* rice genome reveals synergistic contributions of active transposable elements and segmental duplications to rice genome evolution

Kui Li [1], Wenkai Jiang [2], Yuanyuan Hui [1], Mengjuan Kong [1], Li-Ying Feng [3], Li-Zhi Gao [3] ⌂ ⌨, Pengfu Li [1] ⌂ ⌨, Shan Lu [1,4] ⌂ ⌨
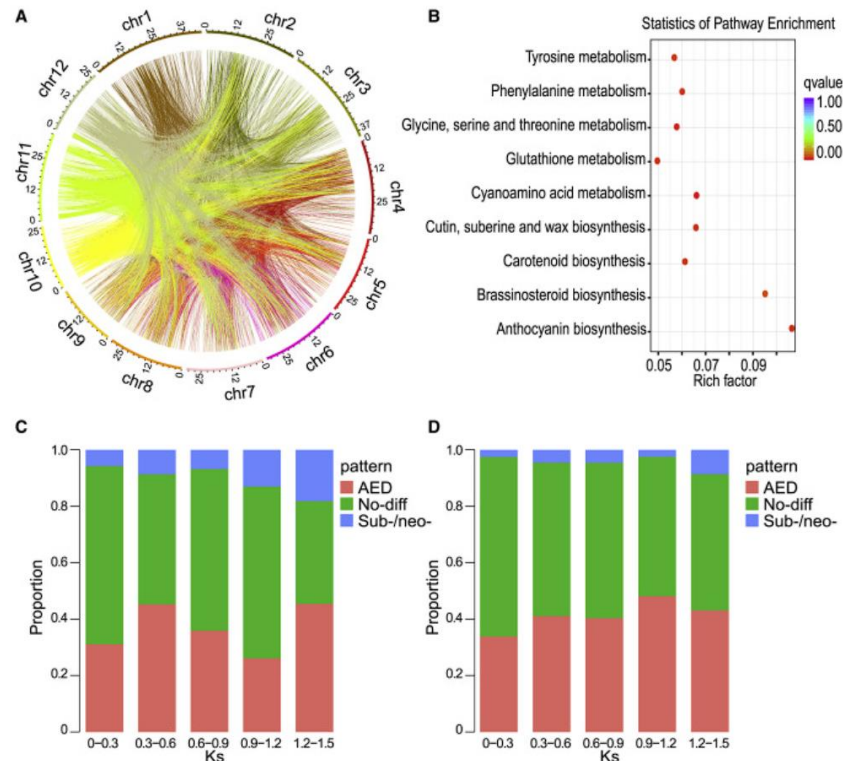
Gapless assembly of maize chromosomes using long-read technologies

Jianing Liu, Arun S. Seetharam, Kapeel Chougule, Shujun Ou, Kyle W. Swentowsky, Jonathan I. Gent, Victor Llaca, Margaret R. Woodhouse, Nancy Manchanda, Gernot G. Presting, David A. Kudrna, Magdy Alabady, Candice N. Hirsch, Kevin A. Fengler, Doreen Ware, Todd P. Michael, Matthew B. Hufford & R. Kelly Dawe ✉
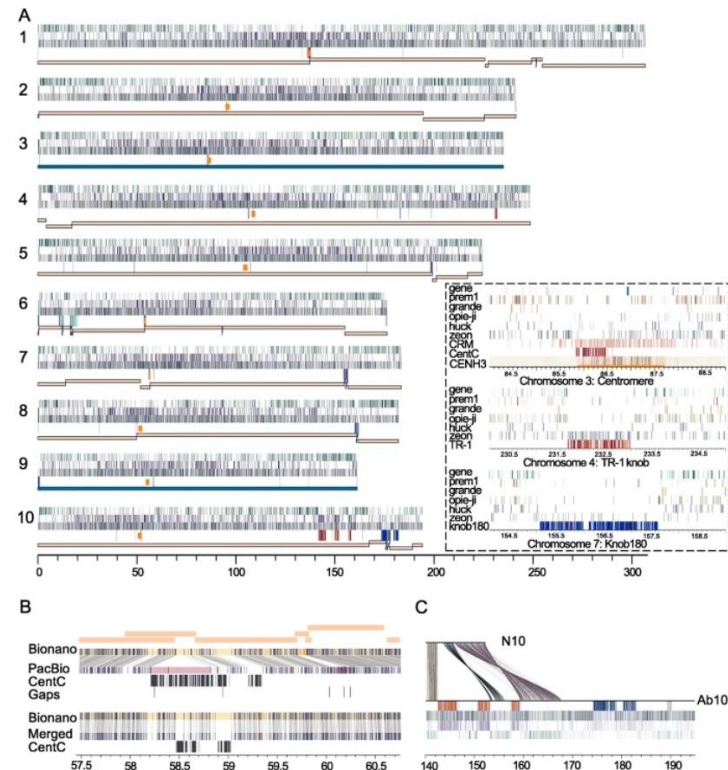
Prospect – Pan genome

Resource

Pan-Genome of Wild and Cultivated Soybeans

Yucheng Liu [1,7,8], Huilong Du [2,7,8], Pengcheng Li [3], Yanting Shen [4], Hua Peng [2,7], Shulin Liu [1], Guo-An Zhou [1], Haikuan Zhang [3], Zhi Liu [1,7], Miao Shi [3], Xuehui Huang [5], Yan Li [6], Min Zhang [1], Zheng Wang [1], Baoge Zhu [1], Bin Han [6], Chengzhi Liang [2,7], Zhixi Tian [1,7,9]
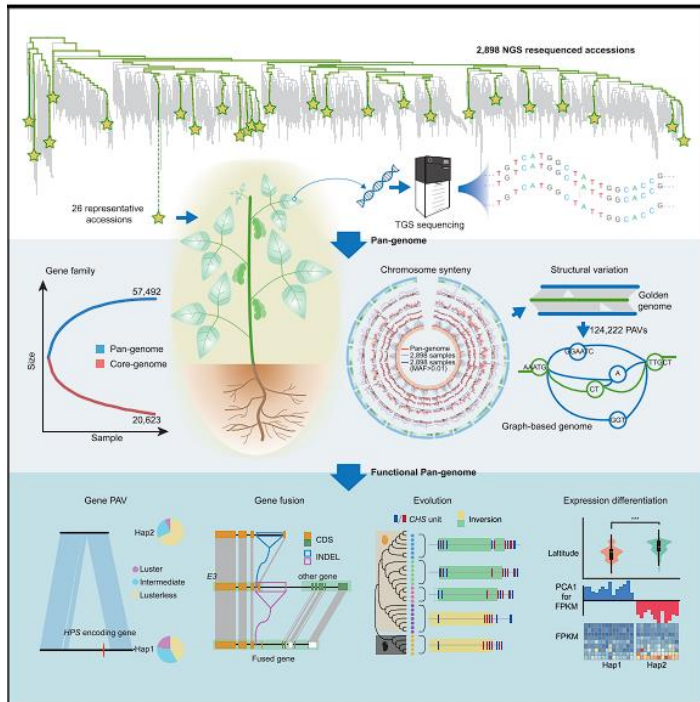
Resource

Pan-genome analysis of 33 genetically diverse rice accessions reveals hidden genomic variations

Peng Qin [1,9], Hongwei Lu [2,3,9], Huilong Du [2,3,4,9], Hao Wang [1,9], Weilan Chen [1,9], Zhuo Chen [2,3,9], Qiang He [4], Shujun Ou [5], Hongyu Zhang [4], Xuanzhao Li [4], Xiuxiu Li [2,3], Yan Li [2], Yi Liao [6], Qiang Gao [2], Bin Tu [1], Hua Yuan [1], Bingtian Ma [1], Yuping Wang [1] … Shigui Li [1,10]
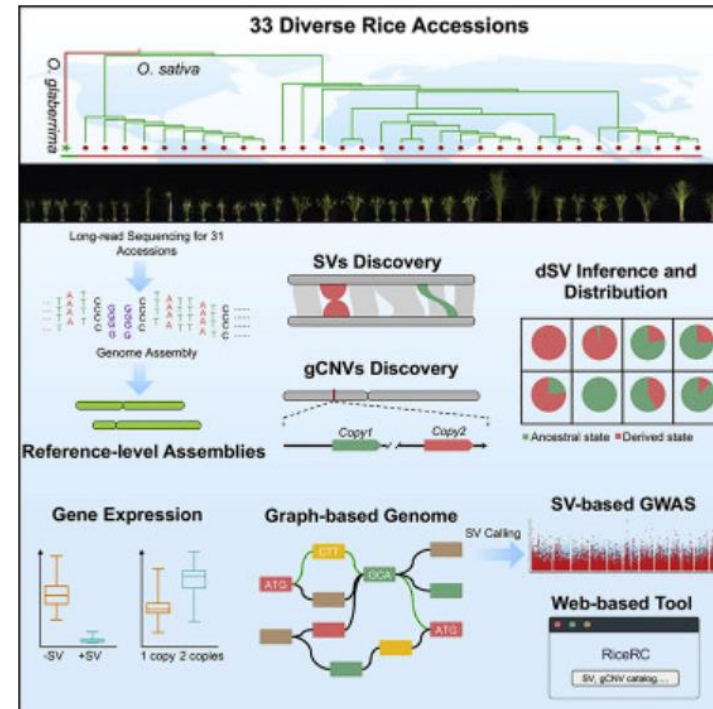
Thanks for your listening!