New Breeding Techniques: Breeding using Natural Tools

Jaroslav Doležel

Institute of Experimental Botany, Czech Academy of Sciences, Center of the Region Haná for Biotechnological and Agricultural Research, Olomouc, Czech Republic

The Agenda for Sustainable Development

2 ZERO HUNGER

END HUNGER, ACHIEVE FOOD SECURITY AND IMPROVED NUTRITION AND PROMOTE SUSTAINABLE AGRICULTURE

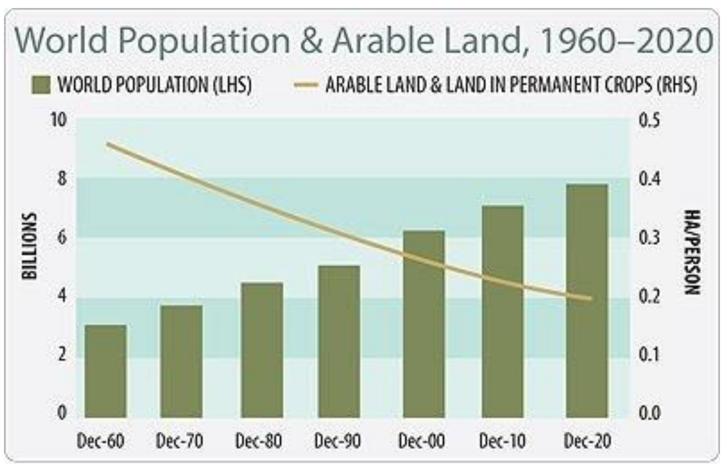
UNITED NATIONS

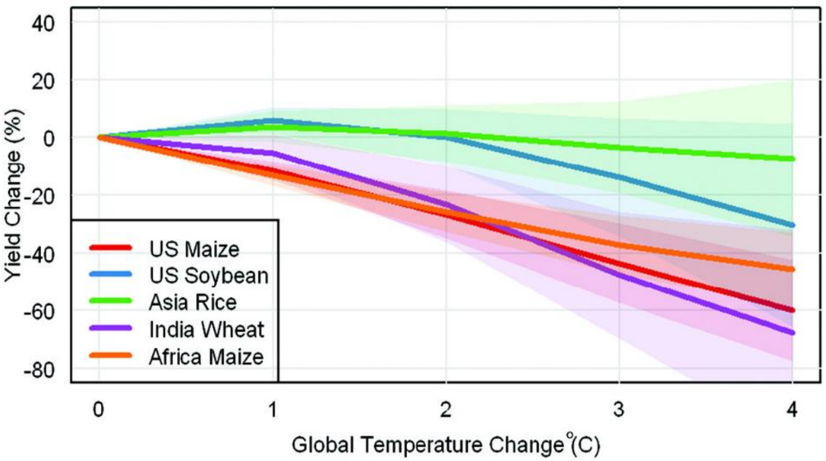
- Food security worldwide is undermined
- An estimated 1 in 10 people worldwide are suffering from hunger
- A staggering 2.3 billion people are moderately or severely food insecure
  - Population growth: 8 billion in 2022, +2 billion expected in 2050
  - Climate change
  - COVID-19
  - Russian invasion to Ukraine
- A need to increase the production of agricultural crops
  - Most of our food comes from plants!

A challenge to produce more

Increase in crop yields per hectare is slower as compared to the population growth rate

- High yields of crops require sufficient moisture, intensive fertilization and the use of herbicides, fungicides and insecticides
- At current levels of productivity, 593 million ha would be missing in 2050 (~2x India)





- Negative impact of climate change (+1.5 °C expected in 2030)

High input agriculture not sustainable

EU Green Deal Targets for 2030

- Reduce by 50% the overall use of chemical pesticides
- Reduce by at least 20 % the use of fertilizers
- At least 25% of the EU's agricultural land under organic farming
- Bring back at least 10% of agricultural area under high diversity landscape features

Production decrease rather than increase

- Euroseeds: Over 23% hectare-weighted production losses can be expected by 2030

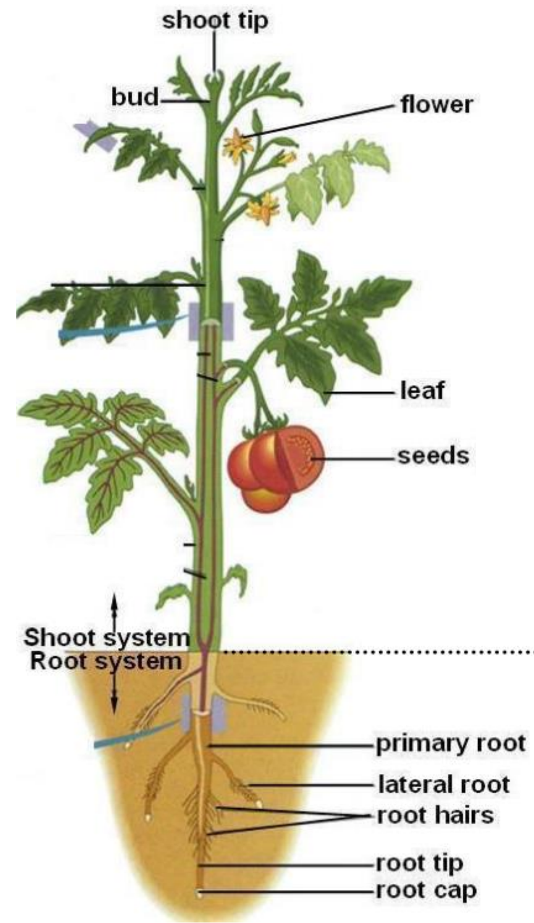
Adapted crops and varieties are needed

- Current crop varieties are not suitable for the low input/high production agriculture

Crops with the new properties

Climate resilient crops with higher and stable yields

- The ability to maintain or increase yields under stress conditions
  - Drought
  - Flooding (submergence)
  - Heat
  - Chilling
  - Freezing
  - Salinity
- Enhanced tolerance to biotic and abiotic stresses



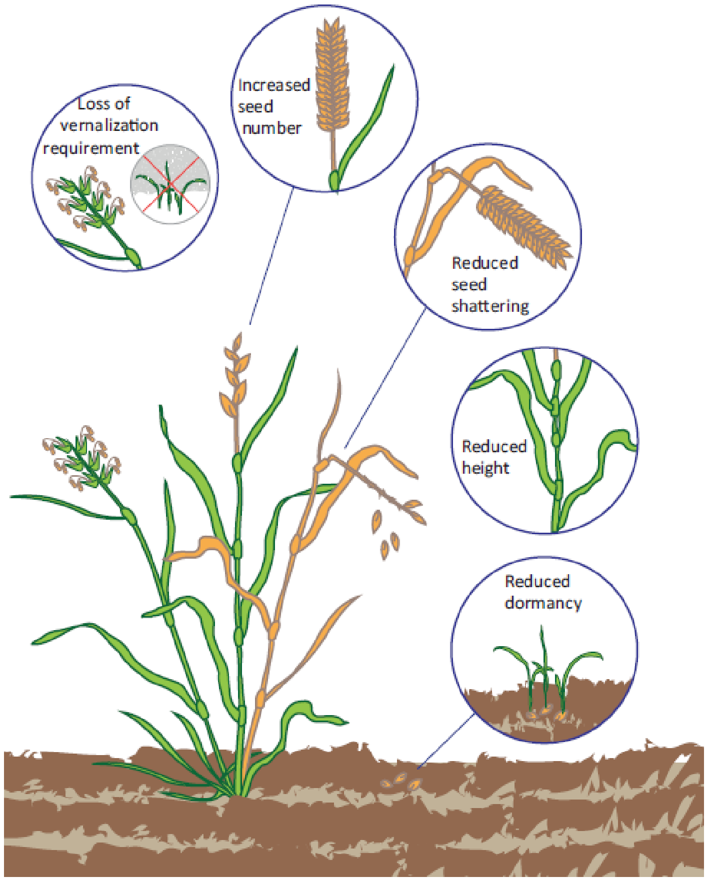
The way to the new properties

- Dramatic changes in plant body organization and in the function of its tissues and organs
- The extent of the changes needed may be compared only to domestication

Plant domestication

Domestication syndrome traits

- Daylength independence
- Lesser/no vernalization
- Loss of seed dormancy
- Determinate growth
- Shorter height
- Gigantism
- Loss or reduction of seed dispersal
- Loss or reduction of toxic compounds
- Larger fruit or grain



TRENDS in Plant Science

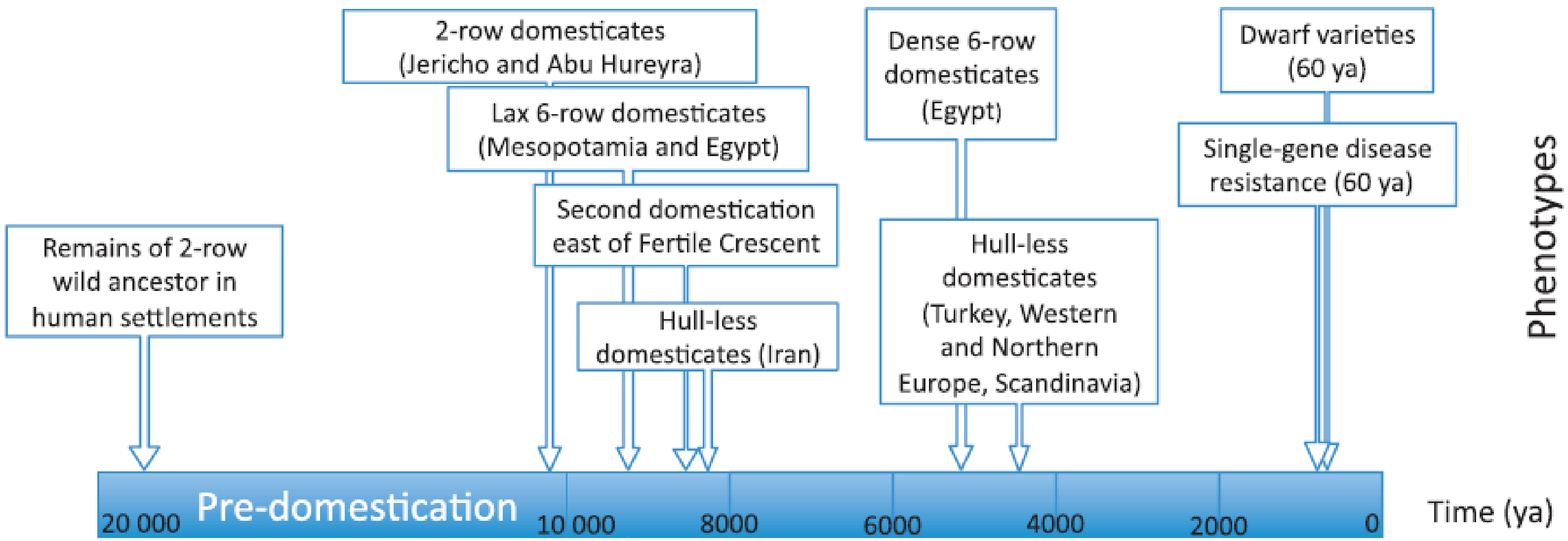
Molecular mechanism

- Spontaneous genome mutations resulting in dramatic changes of a variety of traits

No time for spontaneous mutations

Spontaneous mutations altering traits of interest are rare

- Crop domestication took thousands of years



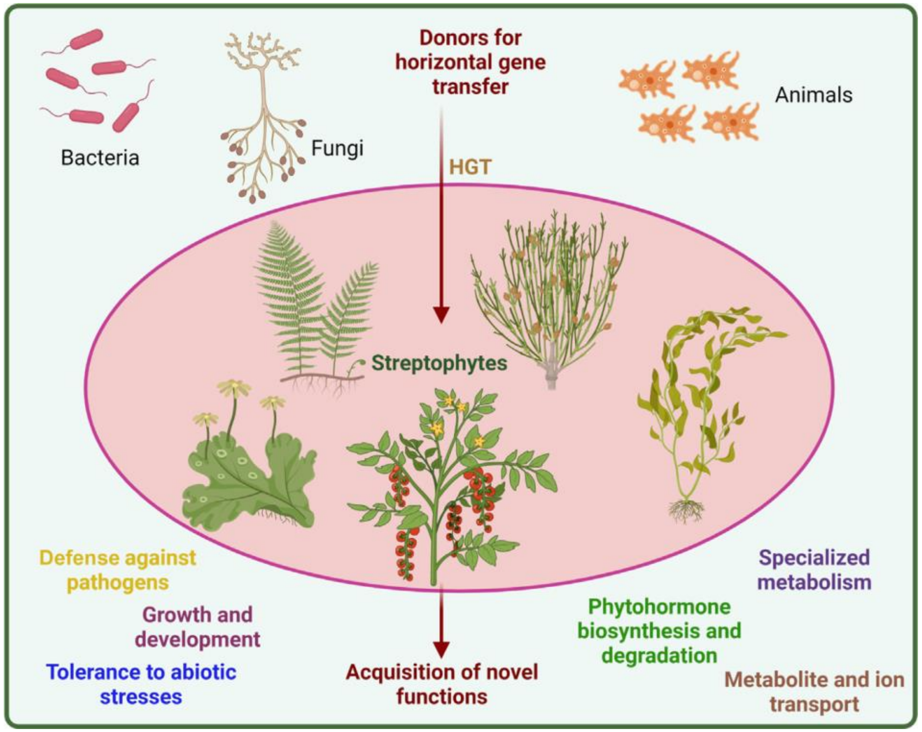
The domestication history of barley

- Mutation breeding suffers from low frequency of desired mutations
  - And a large number of undesirable mutations at unknown loci and with unknown function

Borrowing useful genes

Horizontal (lateral) gene transfer

- The mechanism is natural and by far not rare!
- Important role in the evolution of land plants
  - At least 100 gene families
  - Acquisition of genomic novelty with several distinct novel functions
- Bacteria were the major source of laterally transferred genes followed by fungi



Natural genetic transformation of plants by *Agrobacterium*

- 39 out of 275 dicot species (plants for food, drinks and medicine)

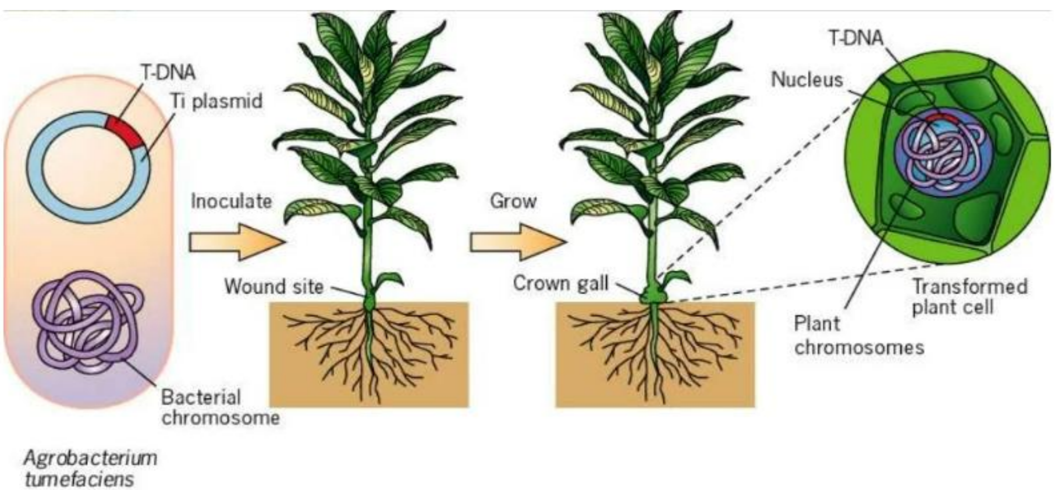
Trends in Plant Science 27: 1203, 2022; Plant Mol Biol. 101: 415, 2019

Gene transfer in crop improvement

Following the nature and exploiting its inventions

- Introduction of the genes of interest either using the natural ability of some bacteria (or by particle bombardment)
- The soil bacterium *Agrobacterium tumefaciens* acquired a mechanism to insert a small segment of its DNA (T-DNA) into the plant cell

Steps involved in Agrobacterium mediated gene transfer

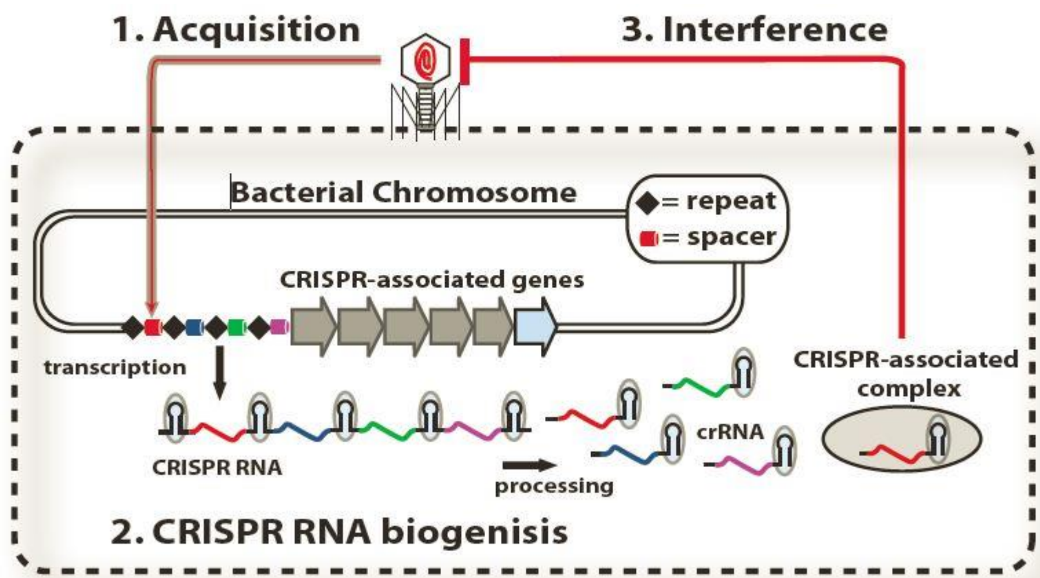


Crown gall formed after infection with *A. tumefaciens*

Performing genome surgery

Self-navigating scalpel developed by the Nature

- Adaptive immunity system found in nearly all archaeal genomes and in about half of bacterial genomes



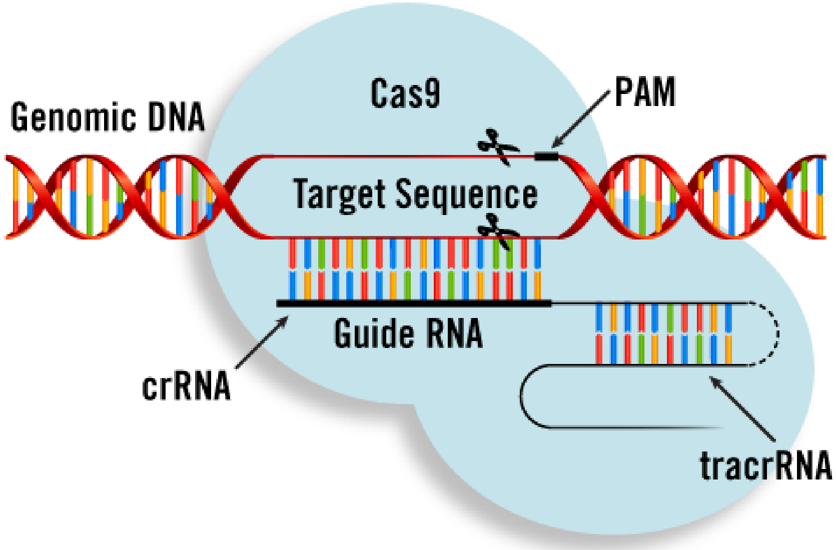
- CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats
- Cas: CRISPR-associated genes

- Repeating sequences of genetic code (CRISPR) are interrupted by remnants („samples“) of genetic code from past invaders
  - This helps to detect and destroy invaders when they return

CRISPR/Cas technique for genome editing

Can be used to precisely target and modify selected genome loci

- Guide RNA directs Cas9 enzyme to the right part of the genome
- Cas9 acts as a pair of ‘molecular scissors’ and cuts DNA



2020 Nobel Prize in Chemistry:
Emmanuelle Charpentier and
Jennifer A. Doudna

- The outcome depends on the way the break in DNA is repaired
  - Gene inactivation; changing gene sequence; altering gene regulation; insertion of DNA sequence of a new gene

New breeding techniques

Depend on modification of the genetic make-up of plants

- Cutting and modifying the genome during the repair process
  - Zinc finger nuclease, TALENs, and CRISPR/Cas
- Genome editing to change on or a few DNA base pairs
  - Oligonucleotide-directed mutagenesis (ODM)
- Transferring a gene from unrelated species
  - Transgenesis
- Transferring a gene from an identical or closely related species
  - Cisgenesis
- Transferring a set of regulatory instructions from same species
  - Intragenesis
- Altering gene activity without altering the DNA itself
  - Epigenetic modification
- Grafting of unaltered plant onto a genetically modified rootstock

The use of GM crops (>500 GM varieties)

GM crops are grown on 14% of cultivated land (190 million ha)

- The way genetic information was modified is documented
- Not a single case of human health damage

- Increased yield, improved product quality and new properties
- Reduced agrochemical use (by 8.6% worldwide)
- Reduced greenhouse gas emission (2018 by 23 million tons of CO_2)
- Higher profit for growers (US$225 billion over 23 years)

Over 500 applications of CRISPR/Cas

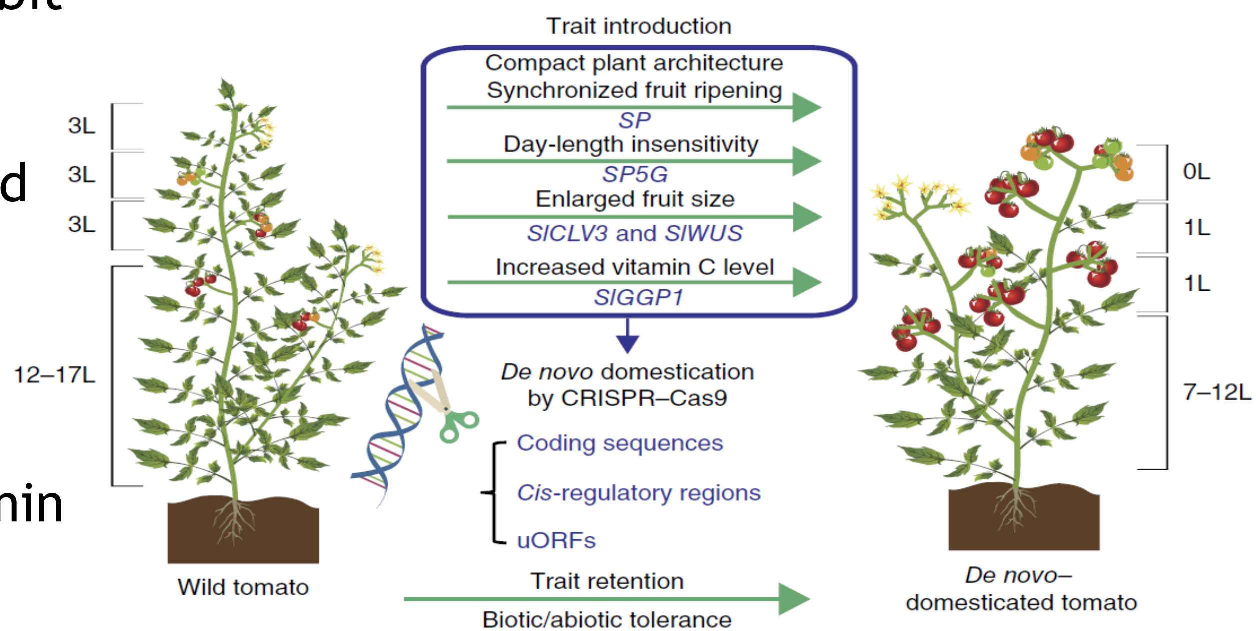
- Wheat, which is not prone to pre-harvest sprouting (inactivation of *Qsd1* gene, which controls seed dormancy)
- Wheat resistant to powdery mildew(inactivation of *Mlo* gene, which codes for a transmembrane protein)
- Wheat with reduced immunogenic gluten proteins (alpha-gliadin gene editing)

- Tomatoes containing provitamin D3 (inactivation of the *7-DR2* gene, which otherwise converts provitamin D3 into other compounds)

https://www.eu-sage.eu/index.php/genome-search

De novo („high-speed“) domestication

- An alternative approach to crop improvement
- De novo domestication of tomato as a successful example
- Multiplex gene editing
- *Solanum pimpinellifolium* (salinity and disease resistance)
  - Editing 5 genes (*SP*, *SP5G*, *SlCLV3*, *SlWUS*, *SlGGP1*)
  - Compact habit
  - Photoperiod neutral
  - Synchronized fruit ripening
  - Larger fruits
  - Higher vitamin C content



Li et al., Nature Biotechnol. 36: 1160, 2018

Conclusions

- Achieving food security in a sustainable manner will require development of a new generation of agricultural crops
- These efforts will require trait modifications on a scale comparable only to domestication
- The challenge is to accomplish this within tens of years as compared to thousands of years of domestication
- The most promising strategy is to leverage the naturally evolved molecular mechanisms, such as bacterial immune system for precision genome editing
- This should facilitate development of climate resilient crops with higher and stable yields