# Improving Crops Using Biotechnological and Genomic Resources

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North Carolina State University

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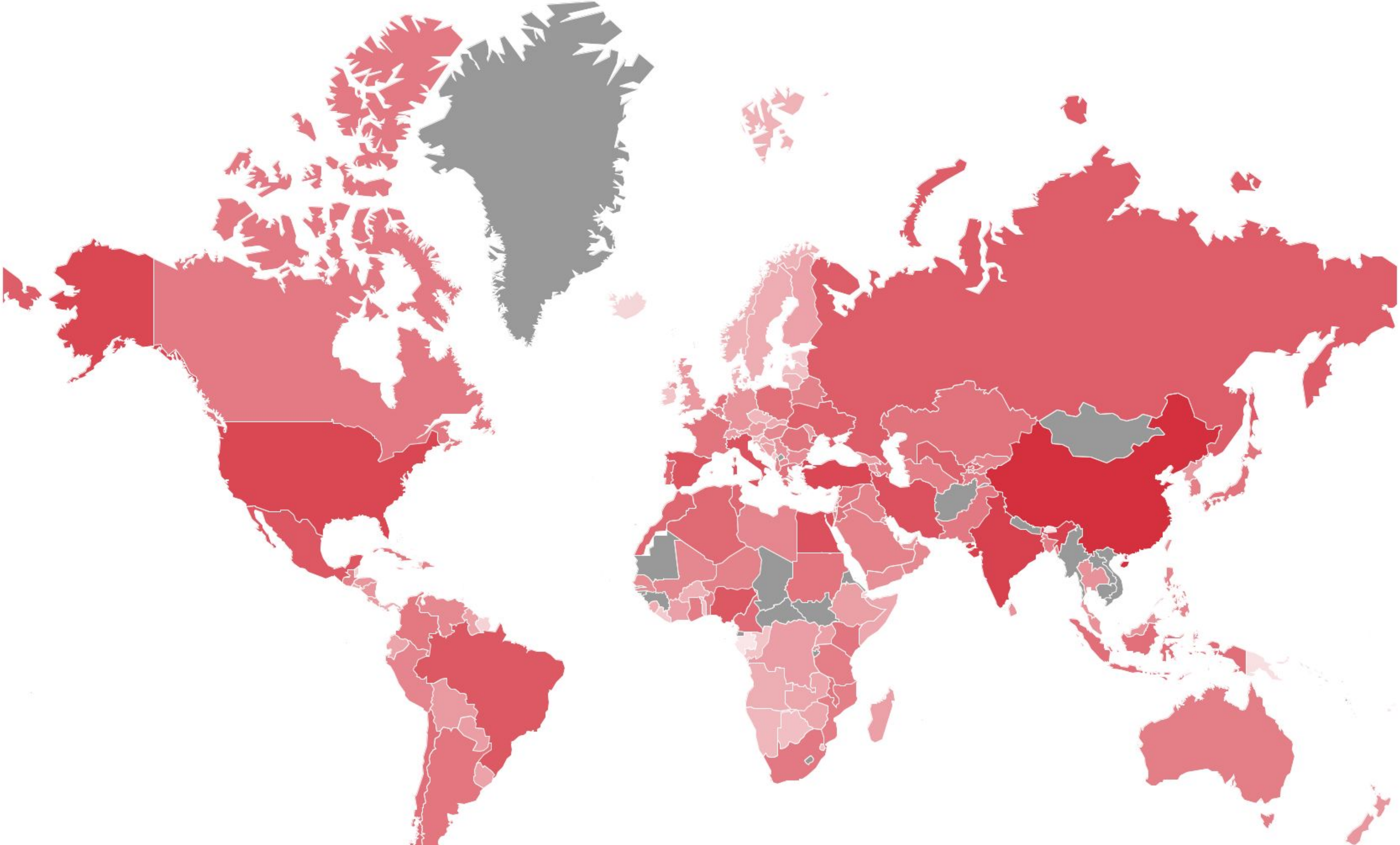
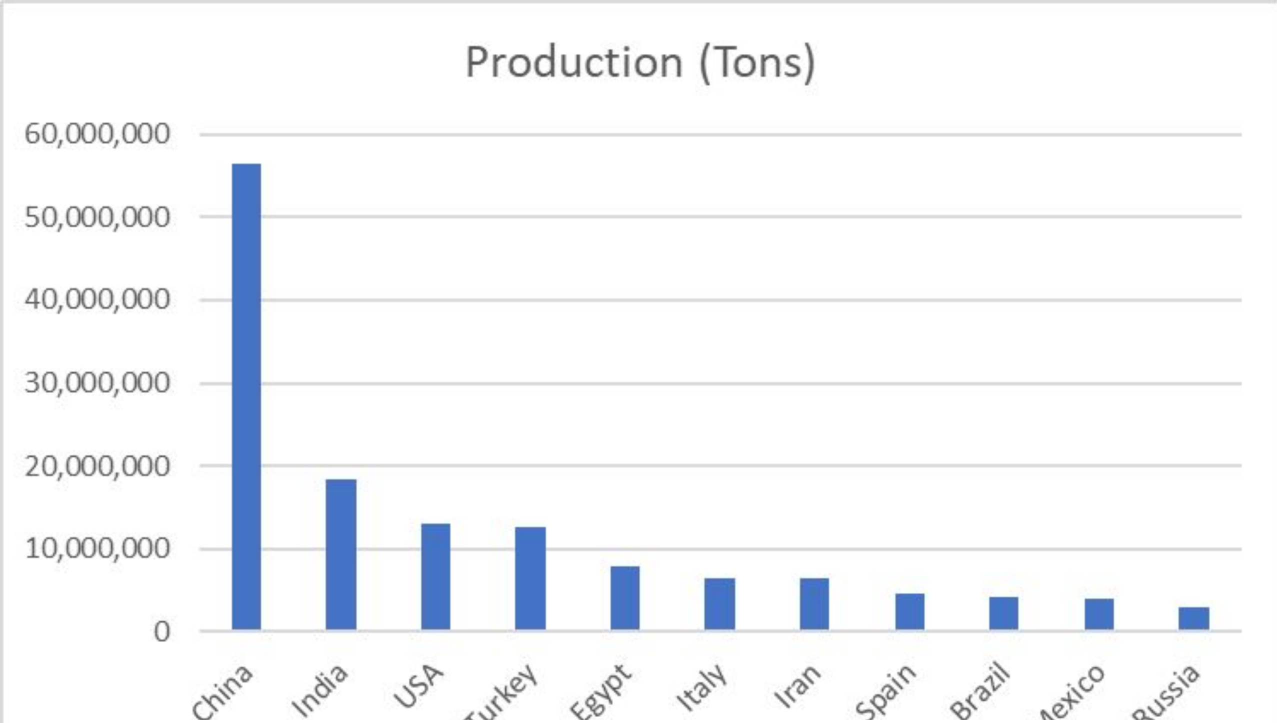
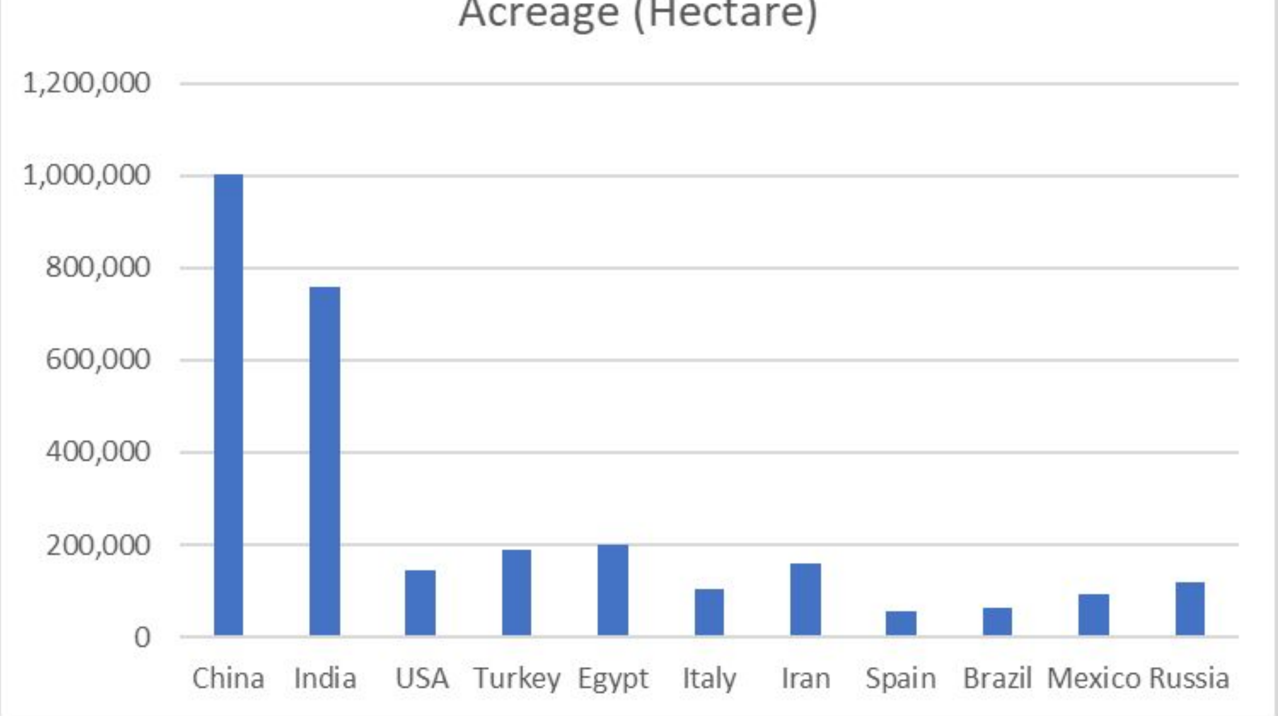
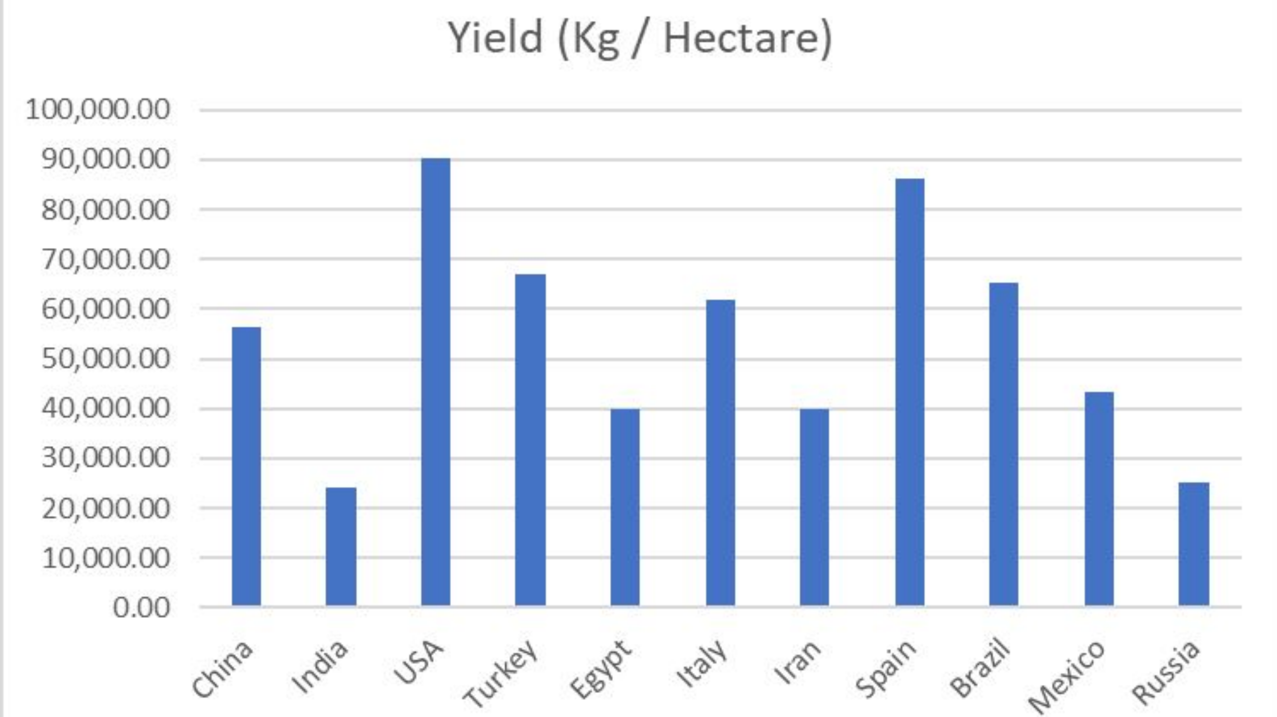
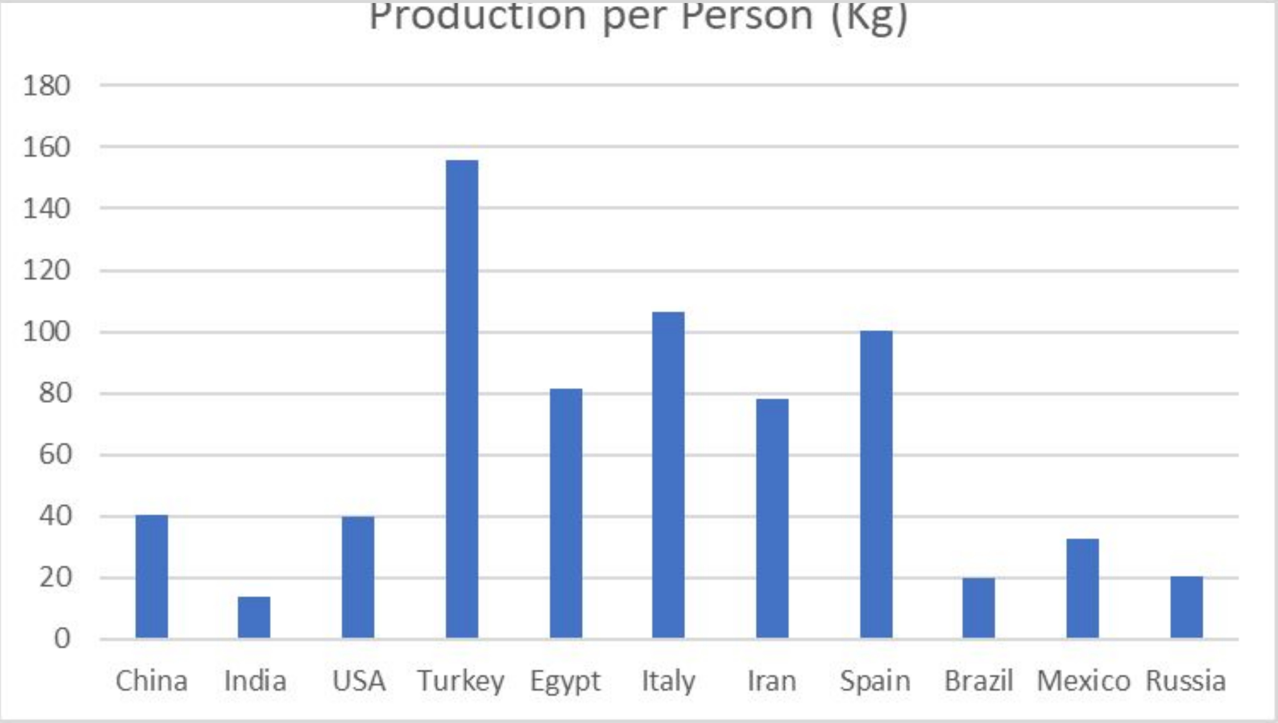
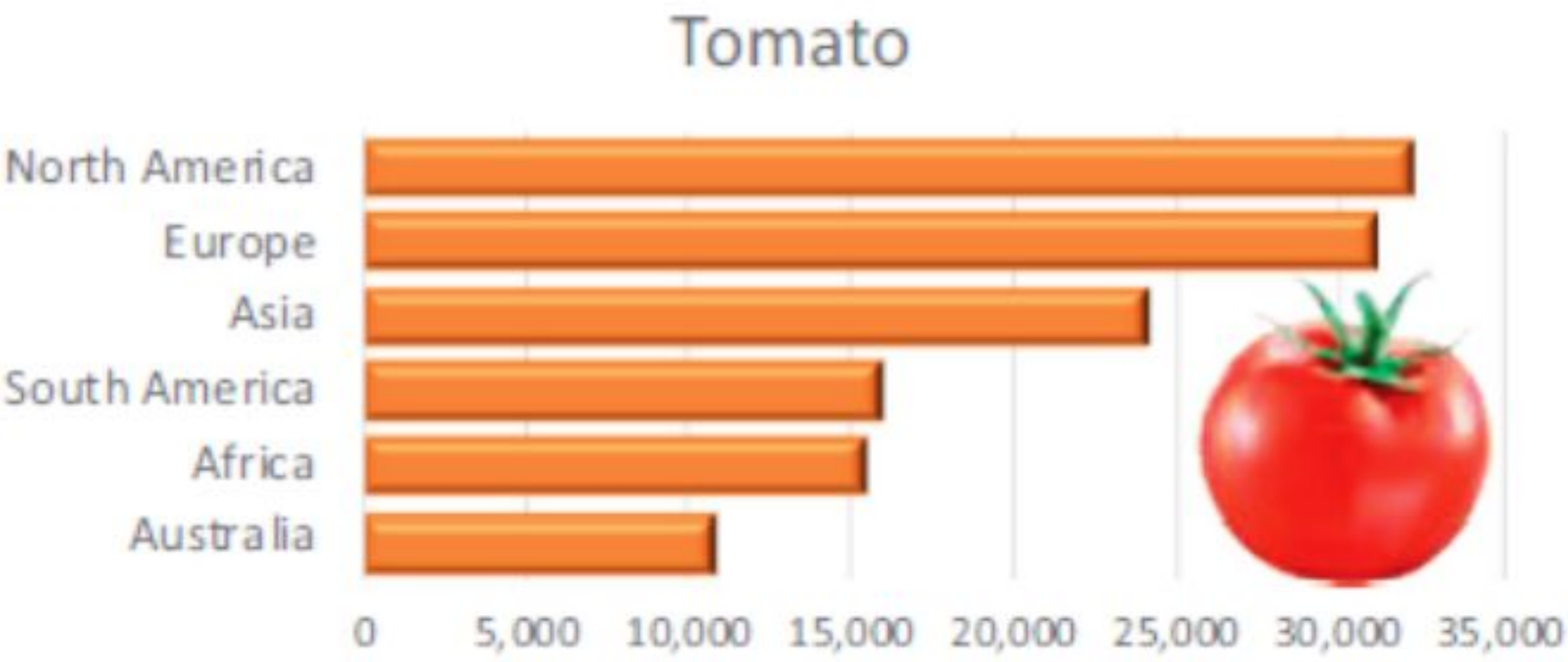



Production (Tons)
60,000,000
50,000,000
40,000,000
30,000,000
20,000,000
10,000,000
0
China
India
USA
Egypt
Italy
Iran
Spain
Brazil


Acreage (Hectare)
1,200,000
1,000,000
800,000
600,000
400,000
200,000
0
China
India
USA
Turkey
Egypt
Italy
Iran
Spain
Brazil
Mexico
Russia


Yield (Kg / Hectare)
100,000.00
90,000.00
80,000.00
70,000.00
60,000.00
50,000.00
40,000.00
30,000.00
20,000.00
10,000.00
0.00
China
India
USA
Turkey
Egypt
Italy
Iran
Spain
Brazil
Mexico
Russia


Production per Person (Kg)
180
160
140
120
100
80
60
40
20
0
China
India
USA
Turkey
Egypt
Italy
Iran
Spain
Brazil
Mexico
Russia




Production in tonnes per million population

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(Simko et al., 2021)

# Tomato Production Overview

- Tomato is the second most important world vegetable crop
- In 2018, tomato production worldwide reached nearly 182 million metric tons and US$ 47.7 billion gross production value (FAOSTAT, 2020)

# Tomato Production in the USA

- U.S. fresh-market tomato:
  - Grown in an area - 175,000 ha
  - Production - 11.5 million mt/year
  - Value - $1.4 billion

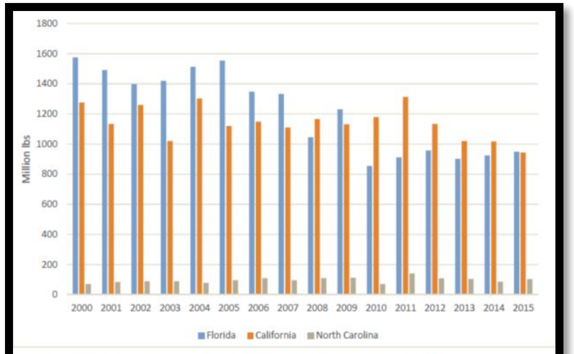



**Figure 2:** U.S. Fresh tomato production in the top three states, 2000 to 2015
Source: USDA, NASS

- California and Florida comprise nearly two-third of total national production.
- North Carolina ranks third nationally representing 3%.
- It is a $32 million industry in NC.

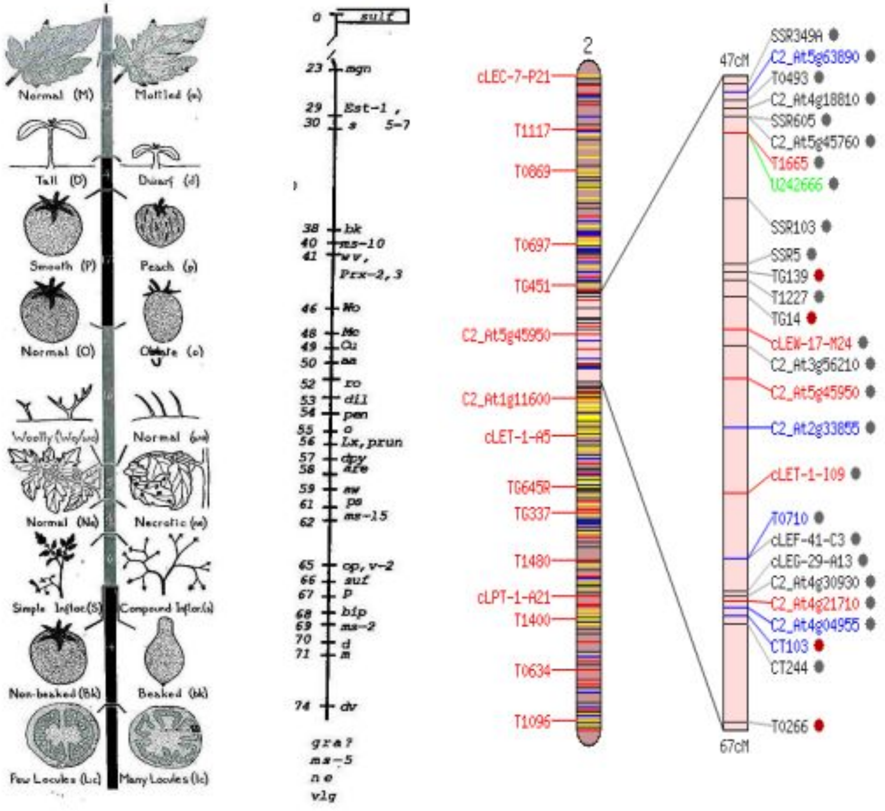
# Program Objectives

- Variety Development
  - Combine disease resistances: bacterial diseases (speck, spot, wilt), fungal (late blight, early blight, septoria leaf spot, fusarium wilt, verticilium wilt), and virus (tomato spotted wilt virus, tobacco mosaic virus)
  - Improve horticultural traits including fruit quality traits, lycopene and flavor
  - Improve heat stress tolerance
- Molecular Research
  - Identify new molecular markers associated with above traits
  - Marker-assisted selection

# Genetic Resources

- Existing tomato genetic resources
- Wild relatives: There are 13 species of *Solanum*, all of which are not cross compatible with *Solanum lycopersicum*.
- The cultivated tomato species is estimated to contain only about 5% of the total genetic variation existing in all tomato species
- Interspecific crosses have been utilized for mapping, and gene identification by using contrasting parents for disease resistance, fruit quality, abiotic stress tolerance and other traits of interest.
- Populations used are F2, BC1, RIL, and NILs

# Report of the Tomato Genetics Cooperative




Volume 59 September 2009

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# Current Mapping Situation

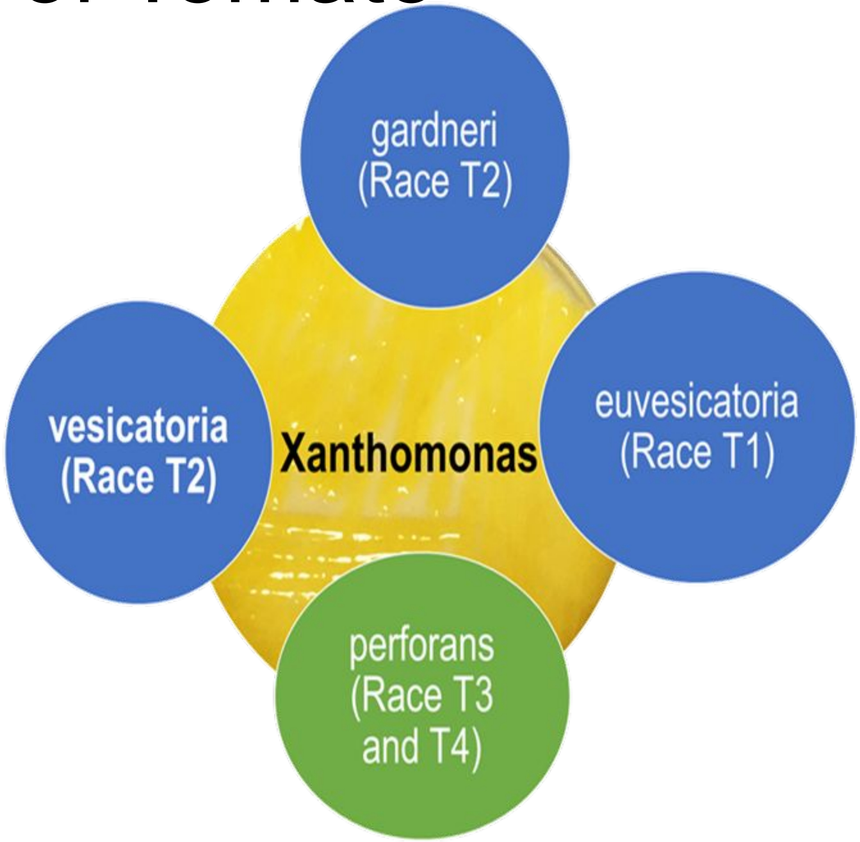
- Development of molecular markers, including AFLPs, SSRs, ESTs, COSs, RAPDs, SCARs, CAPSs, and InDels resulted in the genetic linkage maps with greater marker density.
- High-density genetic map of tomato consisting of 7720 SNP was published using SolCap array by Hamilton et al (2012).
- An “ultra-high density” genetic map developed from *S. lycopersicum x S. pimpinellifolium* RIL population consisting of 141,083 SNP markers (Gonda et al., 2019).

# Genome Sequencing

- First high-quality tomato (cv. Heinz 1706) genomic sequence was published in 2012.
- An improved version of the tomato reference genome assembly (SL4.0) was published by Hosmani et al. (2019)
- The new map has 782 Mbp
- The updated annotation of tomato genome ITAG4.0 reported a total of 34,075 protein-coding genes (Hosmani et al., 2019).
- Pan-genome studies have developed better reference genome and more molecular markers.

# QTL Analysis

# Bacterial Spot of Tomato




- No effective chemical bactericides.
  - ✔ Growers mostly rely on spray of copper and streptomycin.
- No commercial resistant cultivar.

- 17% (late infection) to 66% (early infection) yield losses.

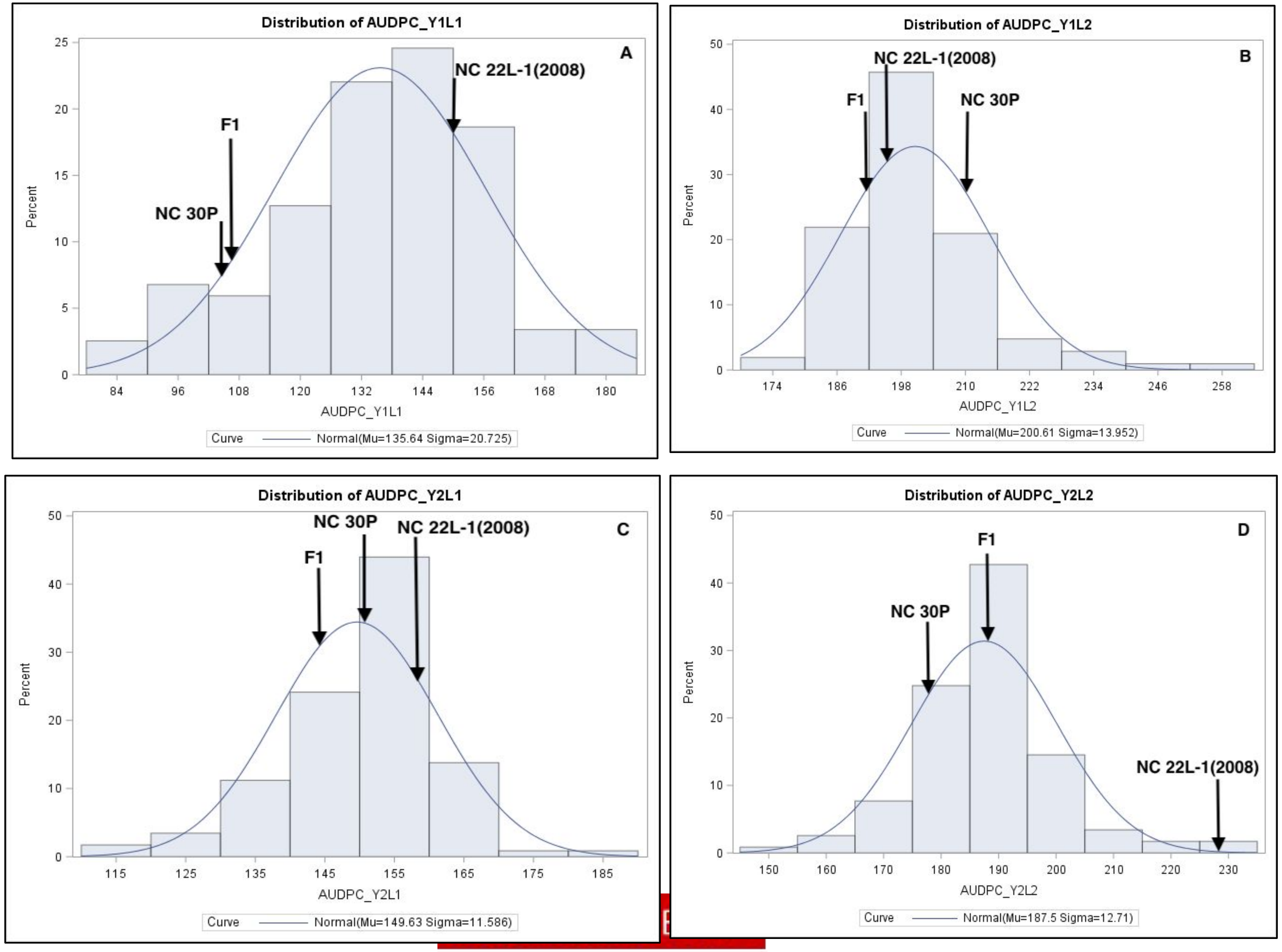
# Phenotypic Distribution

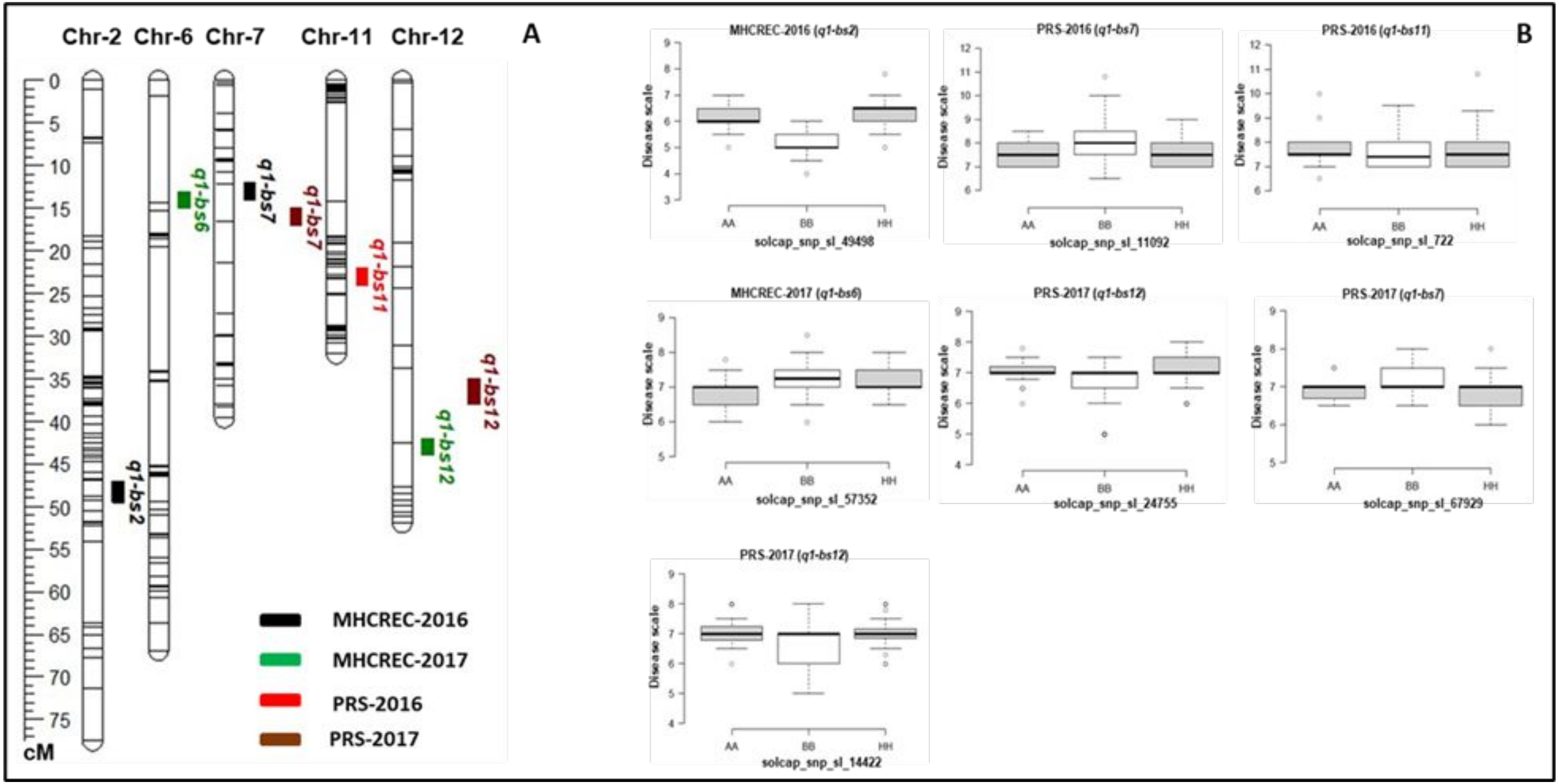
MHCREC-2016

PRS-2016

MHCREC-2017

PRS-2017





A
Chr-2
Chr-6
Chr-7
Chr-11
Chr-12
cM
q1-bs2
q1-bs6
q1-bs7
q1-bs7
q1-bs11
q1-bs12
q1-bs12
MHCREC-2016
MHCREC-2017
PRS-2016
PRS-2017
B
MHCREC-2016 (q1-bs2)
solcap_snp_sl_49498
PRS-2016 (q1-bs7)
solcap_snp_sl_11092
PRS-2016 (q1-bs11)
solcap_snp_sl_722
MHCREC-2017 (q1-bs6)
solcap_snp_sl_57352
PRS-2017 (q1-bs12)
solcap_snp_sl_24755
PRS-2017 (q1-bs7)
solcap_snp_sl_67929
PRS-2017 (q1-bs12)
solcap_snp_sl_14422
Disease scale
AA
BB
HH

Adhikari, P. et al. (2023). Identification of quantitative trait loci associated with bacterial spot race T4 resistance in intra-specific populations of tomato (*Solanum lycopersicum* L.). *PLoSONE* .

| Trait | Chromosome | Flanking markers | Position (cM) | Position (Mb) | LOD | $R^2$ | Additi |
|---|---|---|---|---|---|---|---|
| MECREC-2016 | 2 | solcap_snp_sl_49498 & solcap_snp_sl_49576 | 48.7 | 39-45 | 3.2 | 2.9 | 0.70 |
| PRS-2016 | 7 | solcap_snp_sl_11092 & solcap_snp_sl_67929 | 13.1 | 4-9.5 | 4.4 | 2.5 | -0.38 |
| PRS-2016 | 11 | solcap_snp_sl_722 & solcap_snp_sl_9560 | 23.1 | 39-46.5 | 3.2 | 2.5 | 0.09 |
| MECREC-2017 | 6 | solcap_snp_sl_57352 & solcap_snp_sl_57574 | 14.3 | 42-45 | 3.8 | 21.3 | -0.20 |
| MECREC-2017 | 12 | solcap_snp_sl_24755 & solcap_snp_sl_31628 | 44.5 | 58-64 | 3.1 | 8.2 | 0.27 |
| PRS-2017 | 7 | solcap_snp_sl_67929 & solcap_snp_sl_53393 | 16.6 | 3.8-9 | 6.0 | 3.0 | -0.14 |
| PRS-2017 | 12 | solcap_snp_sl_14422 & solcap_snp_sl_31628 | 36.2 | 60-64 | 3.1 | 7.7 | -0.20 |

| Trait | Chromosome | Flanking markers | Position (cM) | Position (Mb) | LOD | $R^2$ | Additi |
|---|---|---|---|---|---|---|---|
| MHCREC-17 | 1 | solcap_snp_sl_44461 & solcap_snp_sl_43632 | 358.6 | 78-82 | 3.6 | 8.3 | 0.19 |
| MHCREC-17 | 4 | solcap_snp_sl_3106 & solcap_snp_sl_2180 | 59.3 | 55-56 | 5.9 | 9.0 | -0.20 |
| MHCREC-17 | 6 | solcap_snp_sl_24450 & solcap_snp_sl_57594 | 13.7 | 32-42.5 | 10.1 | 23.4 | 0.31 |
| MHCREC-17 | 8 | solcap_snp_sl_21429 & solcap_snp_sl_50211 | 38.4 | 57-61 | 2.7 | 5.6 | -0.16 |
| MHCREC-17 | 9 | solcap_snp_sl_22832 & solcap_snp_sl_58001 | 32.8 | 4.5-8 | 3.4 | 5.0 | -0.15 |
| PRS-17 | 3 | solcap_snp_sl_18985 & solcap_snp_sl_30631 | 58.2 | 48.3-51.7 | 7.3 | 17.8 | 0.22 |
| PRS-17 | 6 | solcap_snp_sl_27215 & solcap_snp_sl_17019 | 28.2 | 42-50 | 3.0 | 6.2 | 0.15 |
| PRS-17 | 7 | solcap_snp_sl_6312 & solcap_snp_sl_6313 | 26.4 | 9-17 | 2.7 | 4.6 | -0.13 |
| PRS-17 | 11 | solcap_snp_sl_56324 & solcap_snp_sl_5970 | 24.1 | 46.5-52 | 3.9 | 7.2 | 0.17 |

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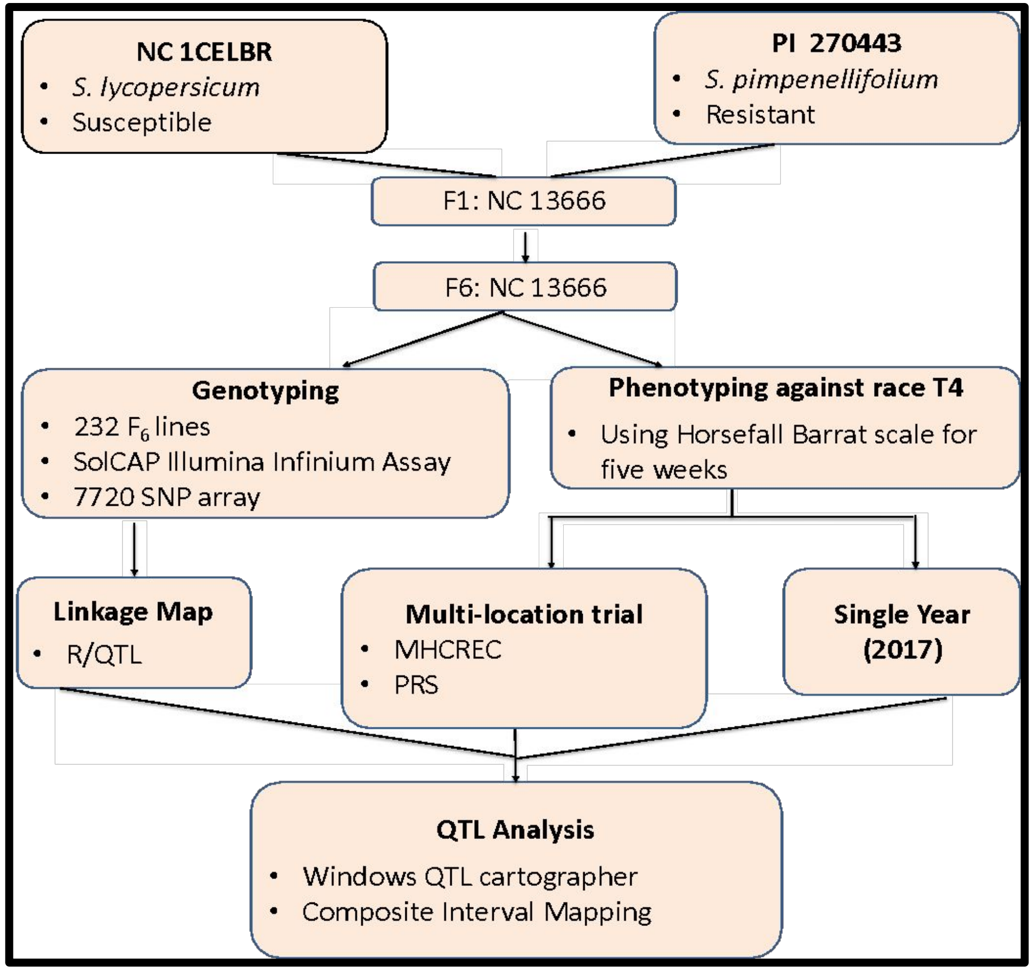
# QTL Validation-NC 13666 Population

**<u>Steps</u>**

1. Generation of mapping population
2. Genotyping
3. Phenotyping
4. Construction of linkage map
5. QTL analysis

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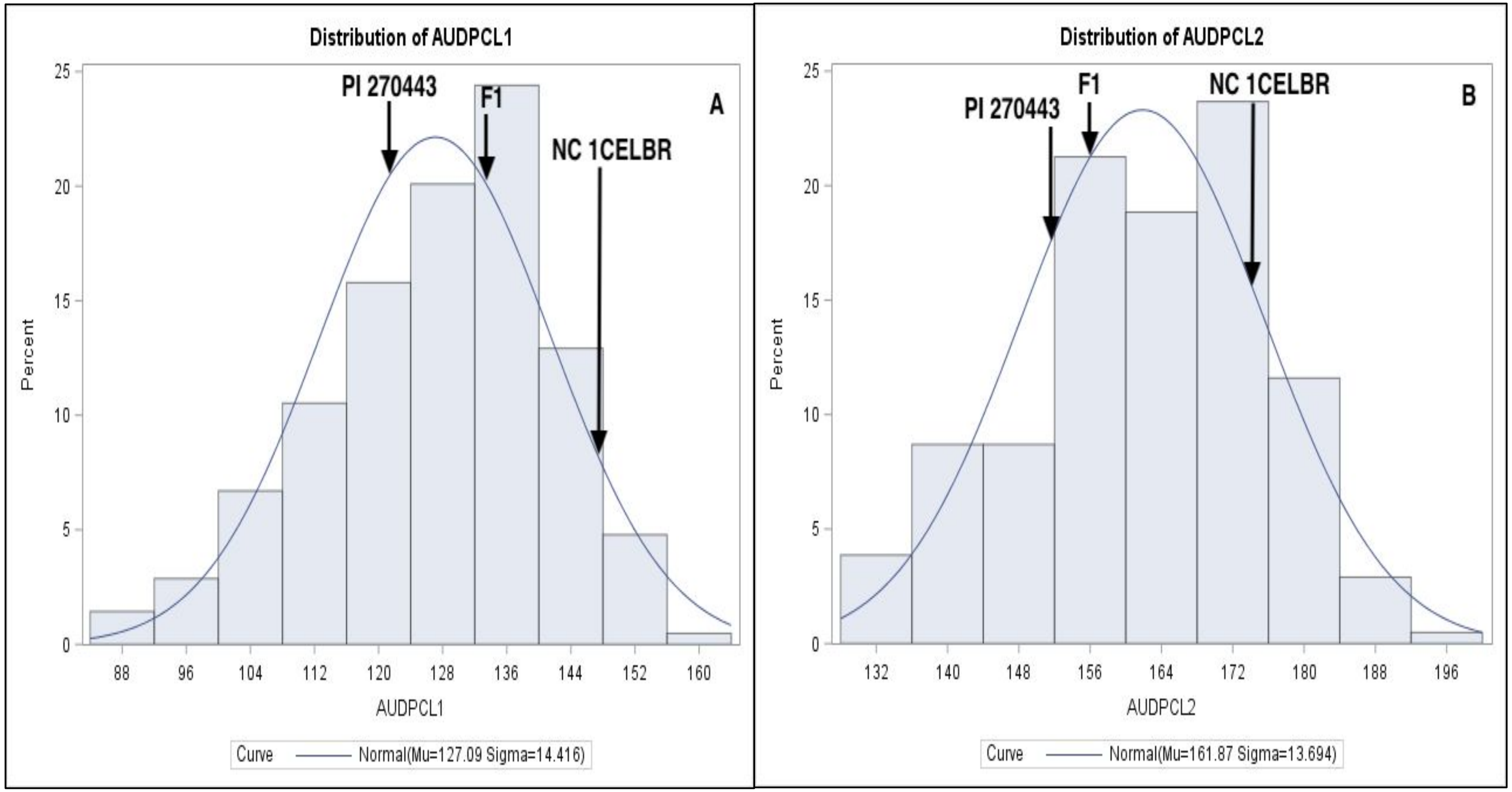
# Phenotypic Distribution

**MHCREC-2017** **PRS-2017**

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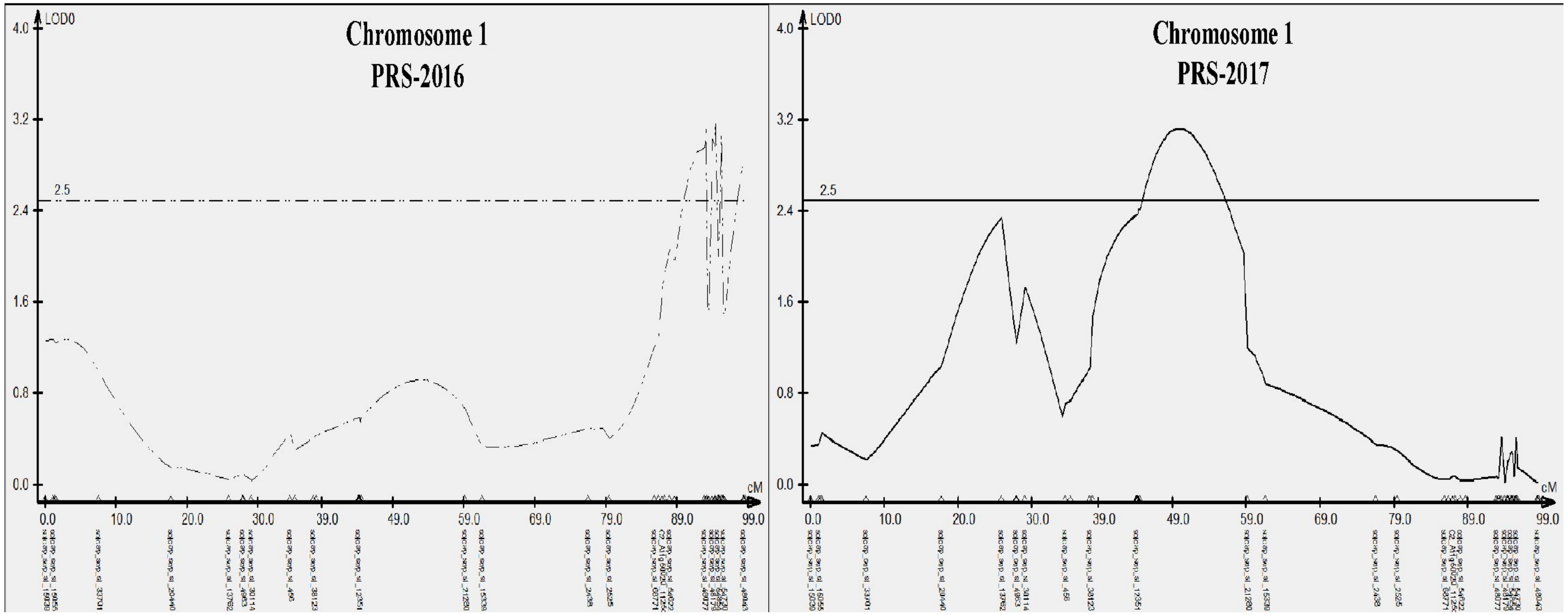
# QTL Validation

| | Physical Map Position (bp) | |
|---|---|---|
| | **NC 10204** | **NC 13666** |
| 1 | 72,658,153 to 88,401,832 (~15Mb) | 74,282,365 to 82,404,532 (~8Mb) |
| 2 | 37554824 to 44069445 (~6Mb) | 44433274 to 46228345 (~1.8Mb) |
| 4 | 60,540,713 to 63,67,2498 (~3Mb) | 55,101,393 to 57,367,706 (~2Mb) |
| 6 | 36,998,358 to 42,288,756 (~5Mb) | 41,150,770 to 43,667,860 (~2.5Mb) |

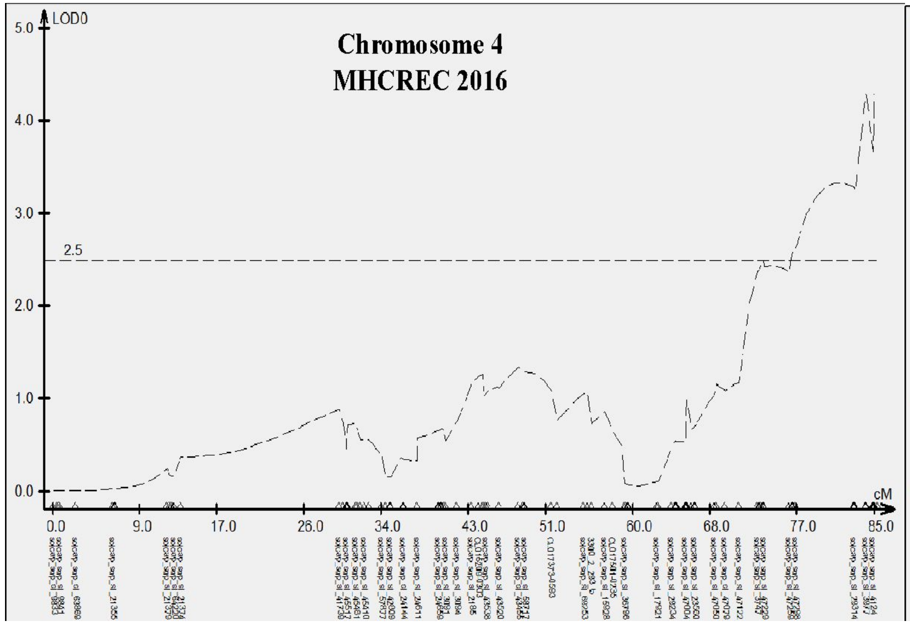
# Bacterial Spot Resistance QTL on Chromosome 1

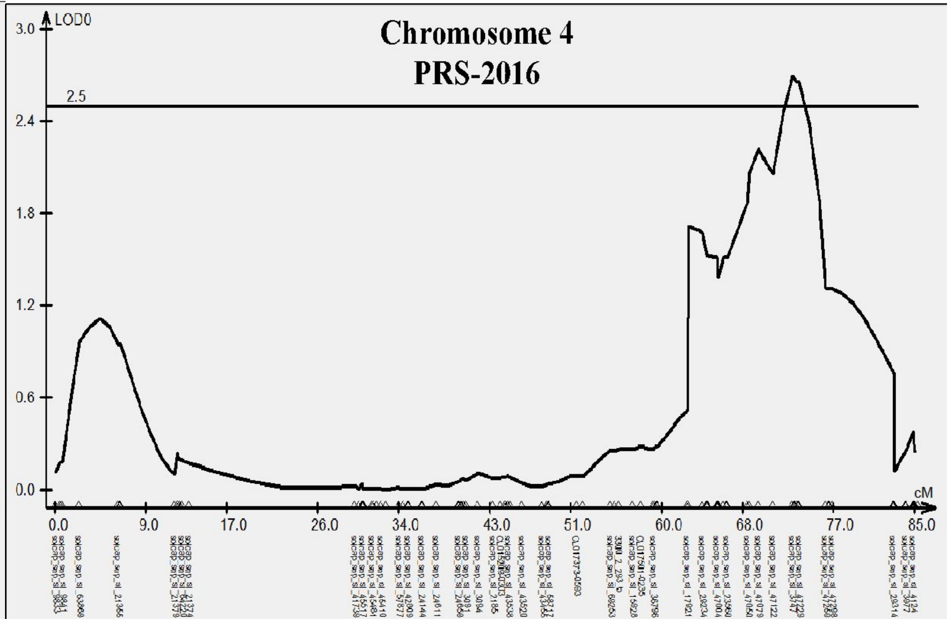



| Environment | Chr. | Marker | Genetic Map Position (cM) | Physical Map Position (bp) | LOD | Additive | $R^2$(%) |
|---|---|---|---|---|---|---|---|
| PRS-2017 | 1 | solcap_snp_sl_12352 & solcap_snp_sl_21280 | 49.58 | 72658153 to 76783695 | 3 | -0.432 | 23 |
| PRS-2016 | 1 | solcap_snp_sl_48181 & solcap_snp_sl_54783 | 95.21 | 88401832 to 87193106 | 3 | 0.395 | 17 |

*Donor of resistance = NC 30P & NC 22L-1(2008)*

# Bacterial Spot Resistance QTL on Chromosome 4

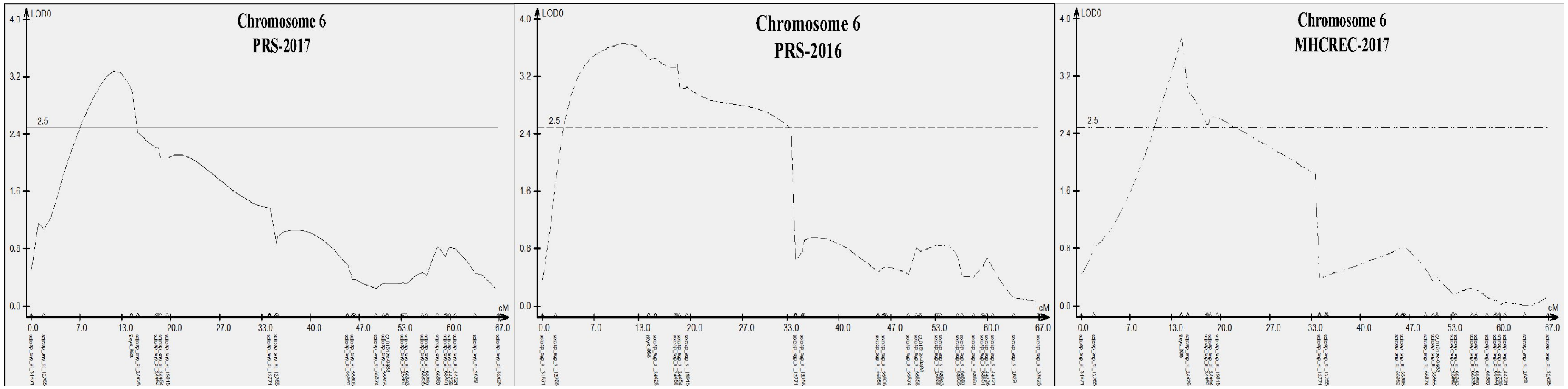



| Environment | Chr. | Marker | Genetic Map Position (cM) | Physical Map Position (bp) | LOD | Additive | $R^2$(%) |
|---|---|---|---|---|---|---|---|
| **PRS-2016** | 4 | solcap_snp_sl_3747 & solcap_snp_sl_47165 | 72.91 | 60540713 to 60552797 | 3 | 0.496 | 15 |
| **MHCREC-2016** | 4 | CL009271-0329 | 84.96 | 63672498 | 4 | 0.522 | 14 |

*Donor of re[illegible] (2008)*

# Bacterial Spot Resistance QTL on Chromosome 6




| Env. | Chr. | Marker | Genetic Map Position (cM) | Physical Map Position (bp) | LOD | a | $R^2$(%) |
|---|---|---|---|---|---|---|---|
| **PRS-2016** | 6 | solcap_snp_sl_12765 & Bcyc_868 | 11.83 | 36998358 to 42288756 | 4 | -9.2 | 26 |
| **PRS-2017** | 6 | solcap_snp_sl_12765 & Bcyc_868 | 11.83 | 36998358 to 42288756 | 3 | -0.4 | 22 |
| **MHCREC-2017** | 6 | Bcyc_868 & solcap_snp_sl_57352 | 14.33 | 42288756 to 42343473 | 4 | -0.3 | 21 |

*Donor of resistance = NC 30P*

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**Position of *RxopJ4*: ~35Mb**

# RNA-seq Analysis

- Bacterial spot-associated genes
- QTL mapping based on RNA-seq data
- Pathway analysis

International Journal of *Molecular Sciences*

MDPI

*Article*

**Transcriptome-Based Analysis of Tomato Genotypes Resistant to Bacterial Spot (*Xanthomonas perforans*) Race T4**

**Rui Shi [1,2] and Dilip R. Panthee [1,*]**

1 Department of Horticultural Science, Mountain Horticultural Crops Research & Extension Center, North Carolina State University, Mills River, NC 28759, USA; rshi@ncsu.edu
2 Department of Crop and Soil Science, North Carolina State University, Raleigh, NC 27695-7620, USA
* Correspondence: dilip_panthee@ncsu.edu

Received: 9 May 2020; Accepted: 1 June 2020; Published: 6 June 2020

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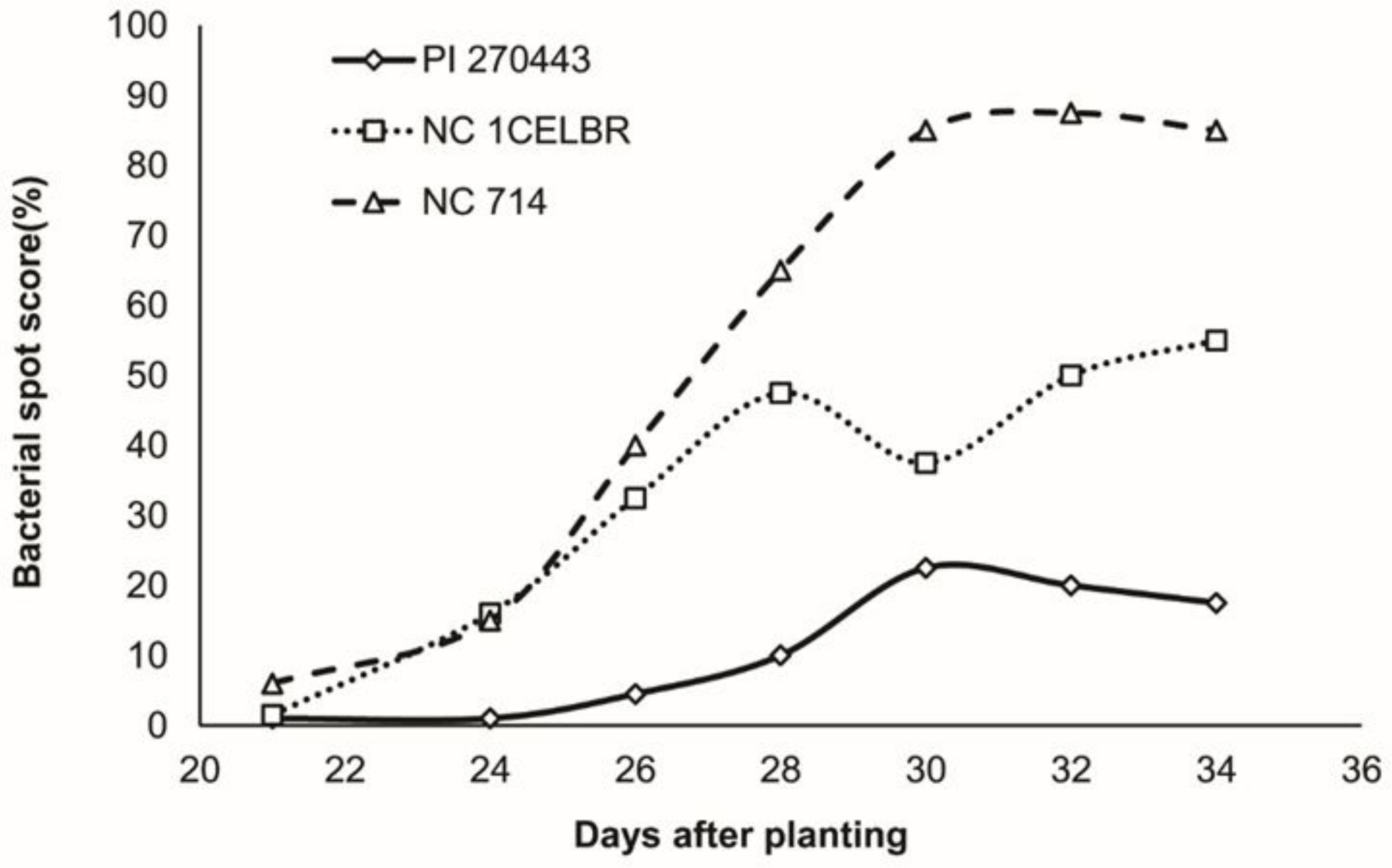
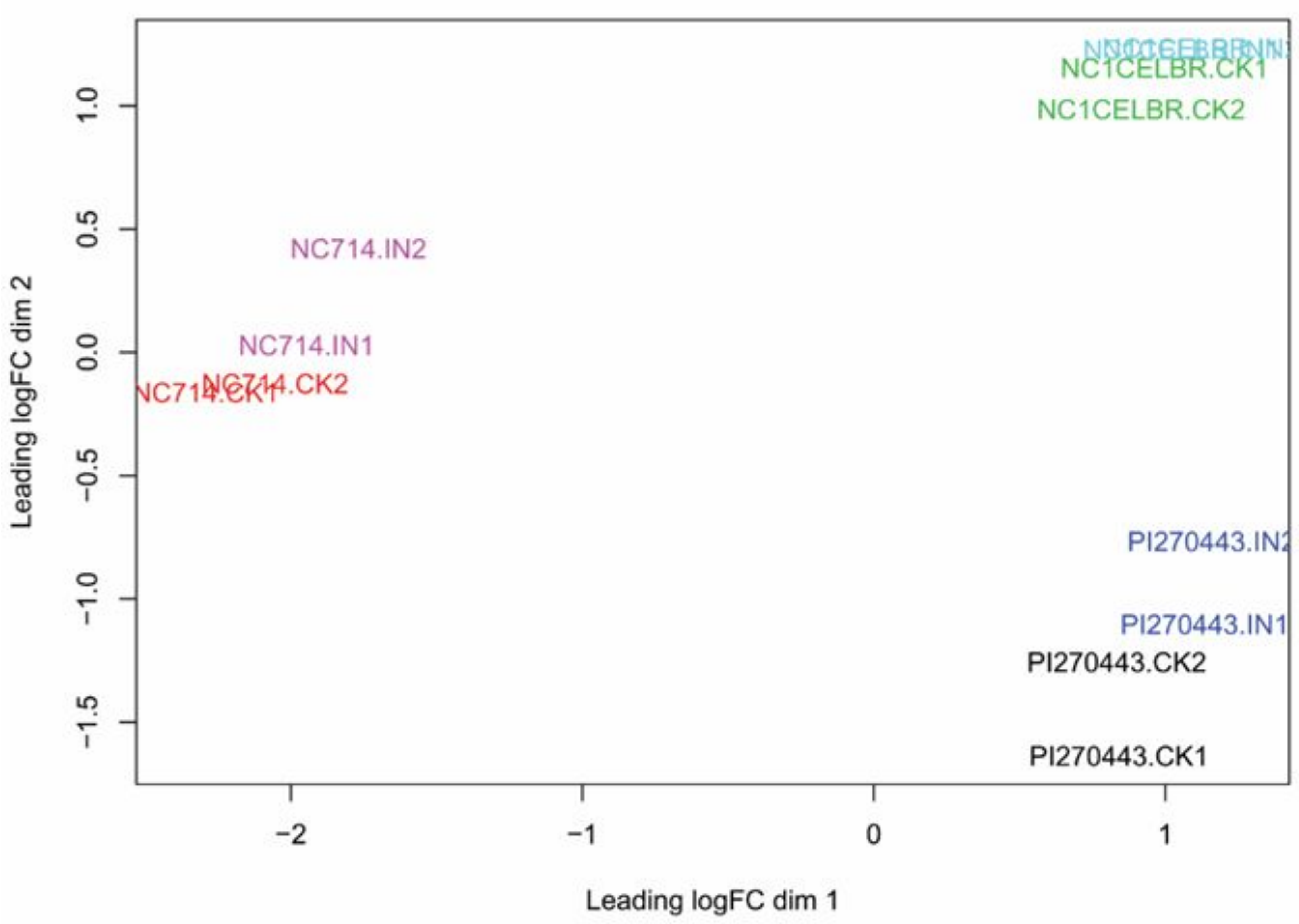
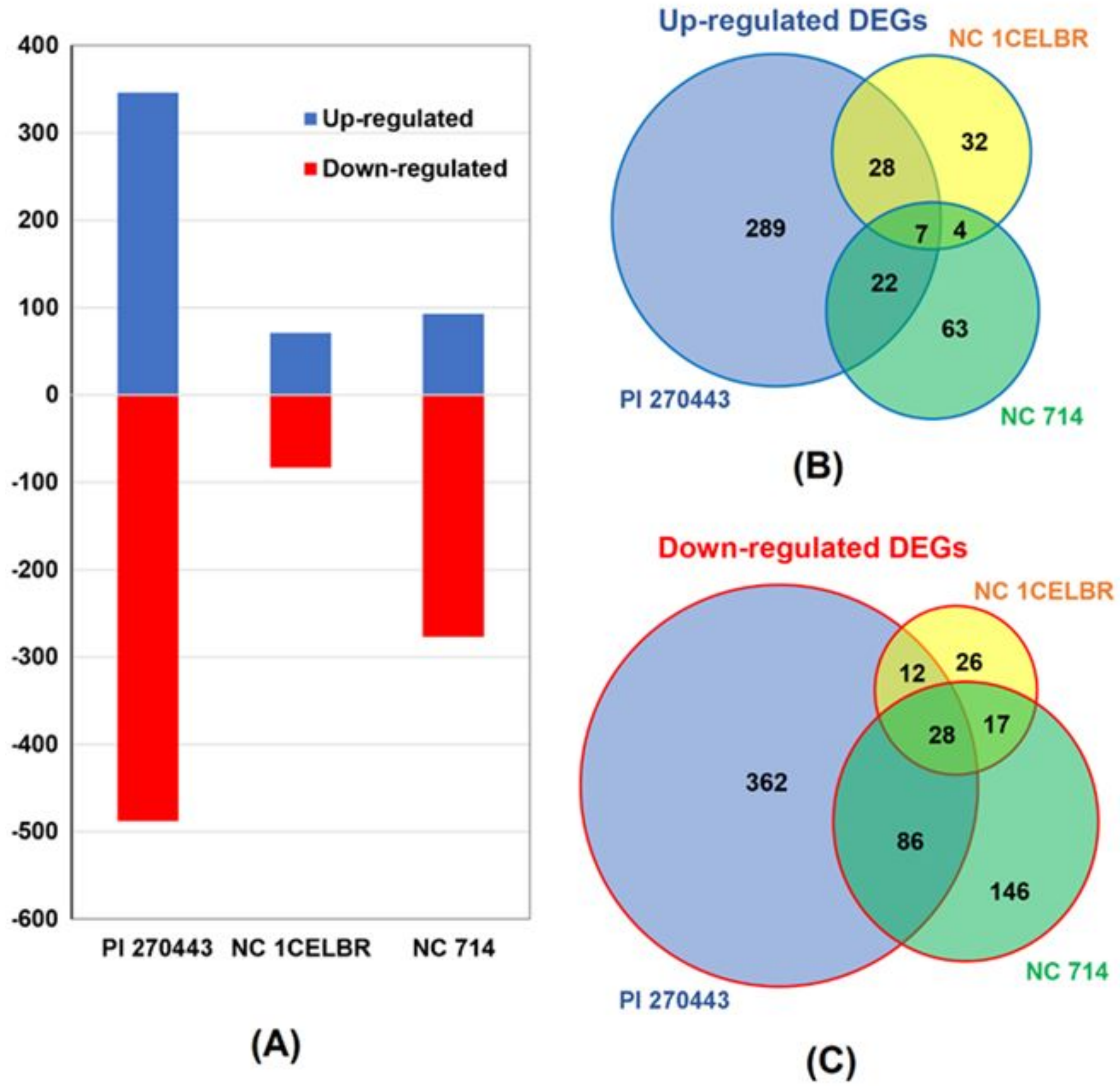
**Abstract:** Bacterial spot (BS) is one of the most devastating foliar bacterial diseases of tomato and is


PI 270443
NC 1CELBR
NC 714
Bacterial spot score(%)
100
90
80
70
60
50
40
30
20
10
0
20
22
24
26
28
30
32
34
36
Days after planting


NC1CELBR.CK1
NC1CELBR.CK2
NC714.IN2
NC714.IN1
NC714.CK1
NC714.CK2
PI270443.IN1
PI270443.CK2
PI270443.CK1
Leading logFC dim 1
Leading logFC dim 2
1.0
0.5
0.0
−0.5
−1.0
−1.5
−2
−1
0
1

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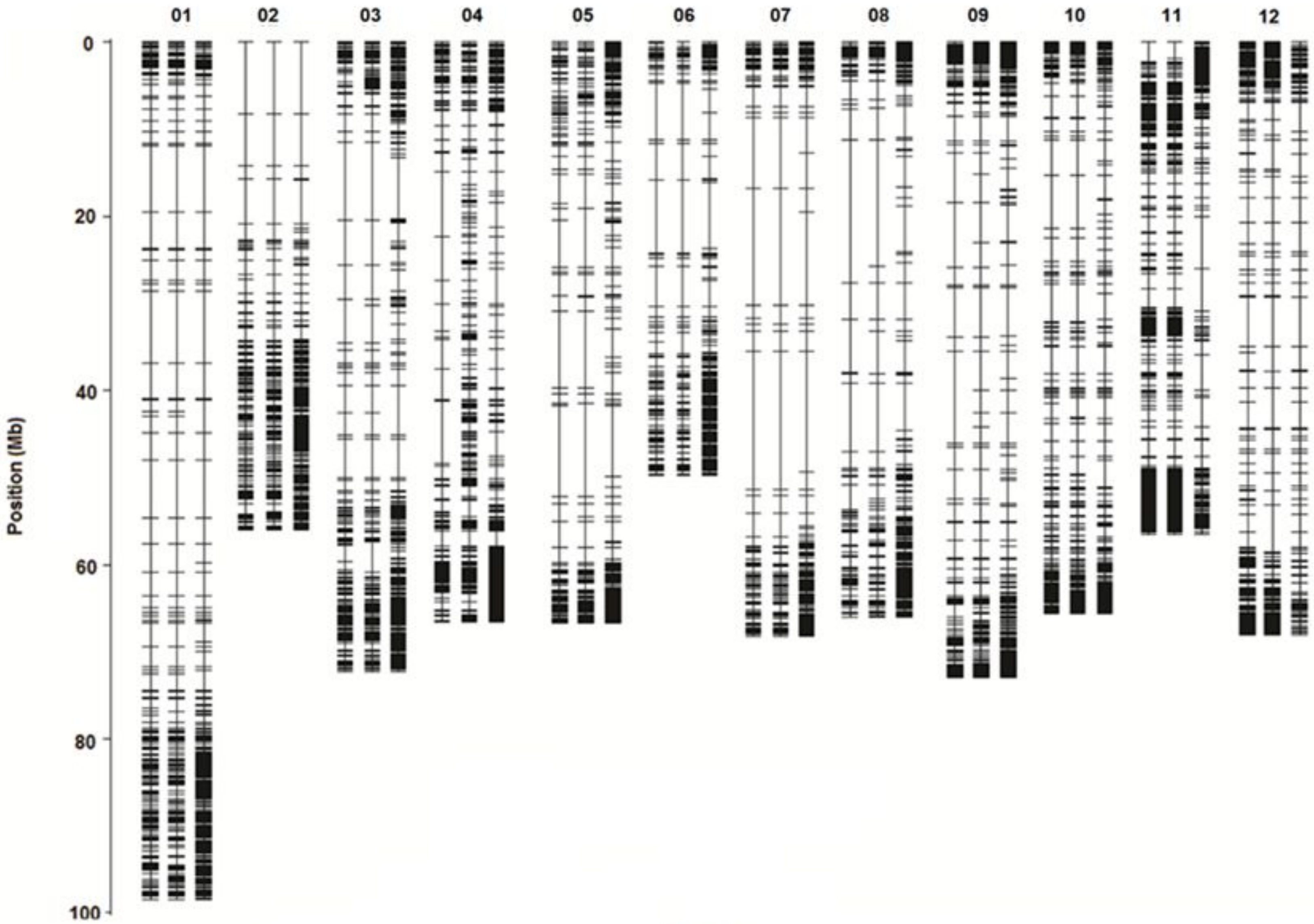


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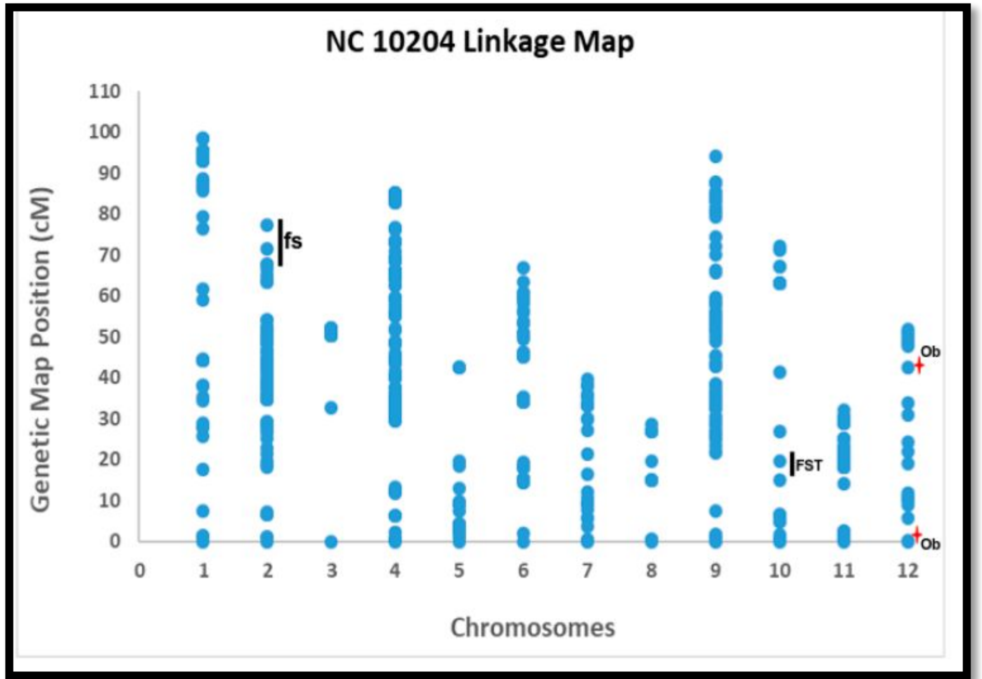
# QTL analysis for fruit quality in tomato


| Chromosome | Markers | Length (cM) |
|---|---|---|
| 1 | 58 | 98.6 |
| 2 | 101 | 77.4 |
| 3 | 60 | 52.1 |
| 4 | 212 | 85.3 |
| 5 | 30 | 42.6 |
| 6 | 60 | 66.8 |
| 7 | 36 | 39.6 |
| 8 | 15 | 28.8 |
| 9 | 138 | 94.1 |
| 10 | 31 | 72.1 |
| 11 | 112 | 30.1 |
| 12 | 33 | 51.9 |
| Total | 886 | 739.4 |




genes
MDPI
Article

# Detection of Quantitative Trait Loci (QTL) Associated with the Fruit Morphology of Tomato

Pragya Adhikari [1], James McNellie [2] and Dilip R. Panthee [3,*]

[1] Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA; adpragya@illinois.edu
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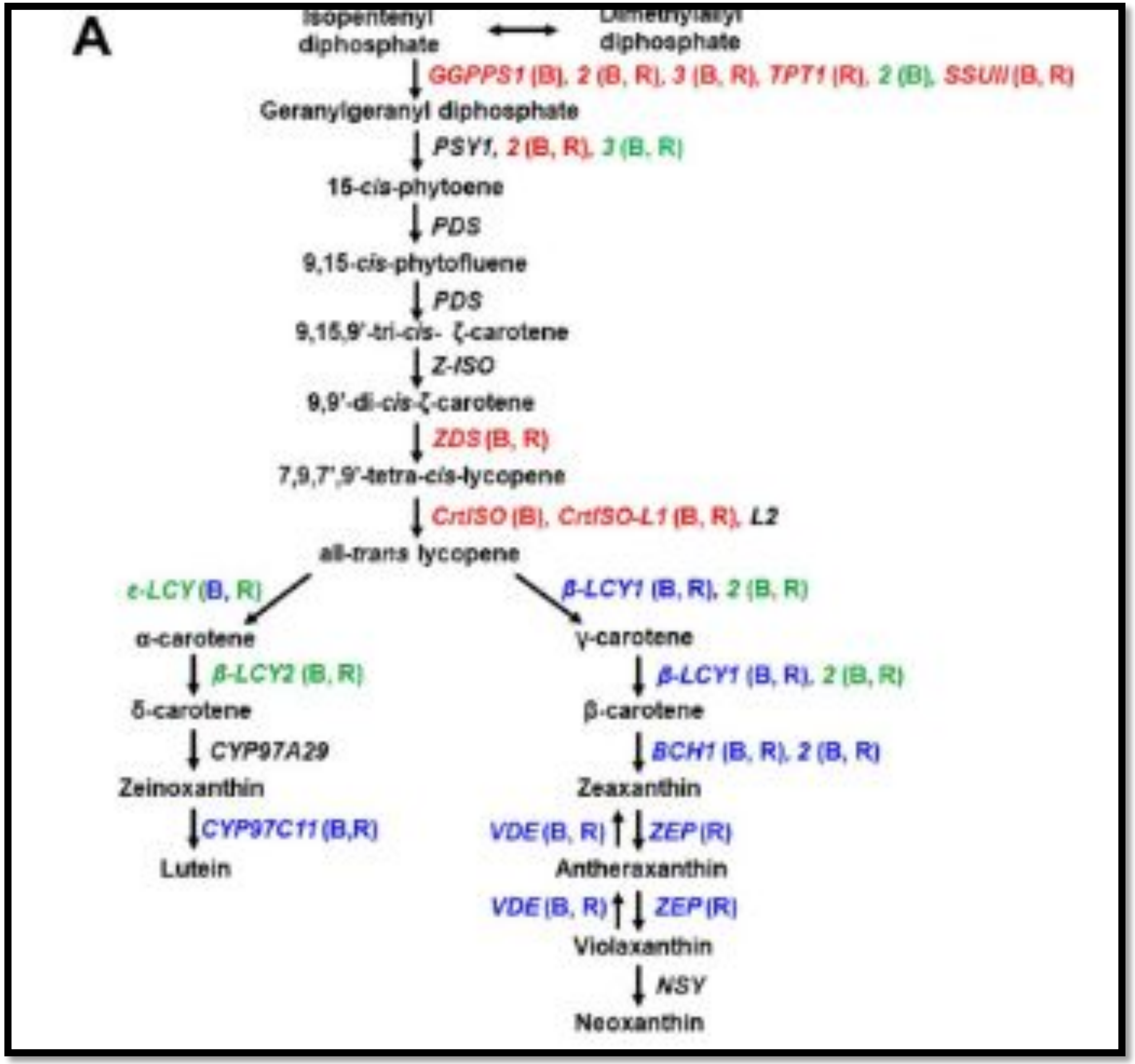
[3] Department of Horticultural Science, North Carolina State University, Mountain Horticultural Crops Research, and Extension Center, Mills River, Mills River, NC 28759, USA
* Correspondence: dilip_panthee@ncsu.edu; Tel.: +1-828-654-8590

| Category of Traits | Traits | Chr. | Closest Markers | Genetic Map Position (cM) | Physical Map Position (bp) | Threshold LOD | LOD Score | Additive Value | R2 (%) | Nature of QTL |
|---|---|---|---|---|---|---|---|---|---|---|
| Fruit Size | Yr1_P | 2 | solcap_snp_sl_21867 | 77.44 | 47,948,927 | 3.7 | 6.9 | −7.76 | 6.9 | [a] consistent; confirmatory |
| | Yr2_P | 2 | solcap_snp_sl_50066 | 67.7 | 44,783,686 | 3.6 | 4.7 | −2.17 | 17.2 | consistent; confirmatory |
| | Yr1_A | 2 | solcap_snp_sl_21867 | 75.44 | 47,948,927 | 3.8 | 7.8 | −228.27 | 8.3 | consistent; confirmatory |
| | Yr2_A | 2 | solcap_snp_sl_42324 | 66.73 | 44,069,445 | 3.7 | 5.4 | −50.58 | 20.0 | consistent; confirmatory |
| | Yr1_WMH | 2 | solcap_snp_sl_21867 | 77.44 | 47,948,927 | 3.9 | 7.7 | −2.23 | 8.8 | consistent; confirmatory |
| | Yr2_WMH | 2 | solcap_snp_sl_21867 | 77.44 | 47,948,927 | 3.7 | 3.7 | −0.72 | 15.8 | consistent; confirmatory |
| | Yr1_MW | 2 | solcap_snp_sl_21867 | 72.44 | 47,948,927 | 3.6 | 6.9 | −0.16 | 8.3 | consistent; confirmatory |
| | Yr2_MW | 2 | solcap_snp_sl_21867 | 76.44 | 47,948,927 | 3.6 | 3.6 | −0.73 | 15.6 | consistent; confirmatory |
| Fruit Shape | Yr1_FST | 10 | solcap_snp_sl_9598, solcap_snp_sl_16517 | 22.77 | 4,260,136;57,327,585 | 3.7 | 4.0 | 0.03 | 10.2 | consistent; novel |
| | Yr2_FST | 10 | solcap_snp_sl_34373, solcap_snp_sl_9598 | 16.07 | 3,991,802;4,260,136 | 3.6 | 5.4 | 0.05 | 24.2 | consistent; novel |
| | Yr1_Ob | 12 | solcap_snp_sl_1573, solcap_snp_sl_58869 | 1.26 | 4,038,812;5,029,856 | 3.7 | 4.9 | −0.10 | 34.0 | [b] not consistent; putative QTL; might be novel |
| | Yr2_Ob | 12 | solcap_snp_sl_24755, solcap_snp_sl_31628 | 43.48 | 7,801,435;64,210,355 | 3.7 | 4.2 | 0.06 | 7.1 | not consistent; putative QTL; might be novel |

Trait: P = perimeter, A = area, WMH = width mid-height, MW = maximum width, FST = fruit shape triangle, Ob = obovoid. [a] QTL that were detected on the close genetic positions (<10 cM) across different generations. [b] QTL that were detected on the different genetic positions (>10 cM) across different generations.



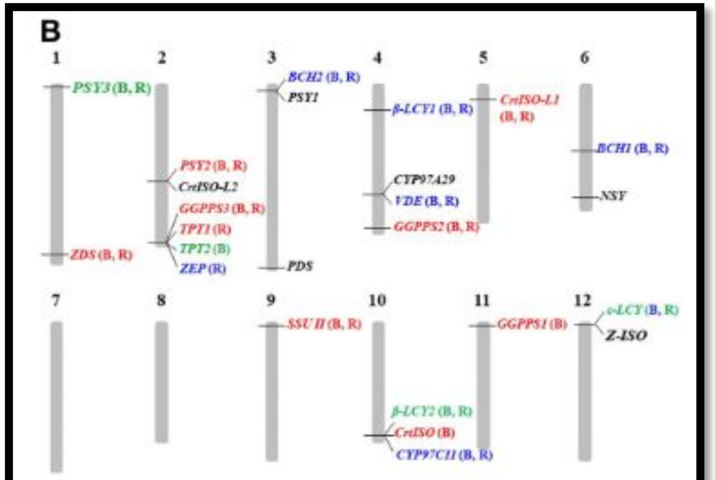




**Figure 7.** The carotenoid biosynthetic pathway in tomato (A) and chromosomal distribution of the 25 carotenoid biosynthesis pathway

Horticulture Research

*Horticulture Research*, 2022, **9**: uhac084
https://doi.org/10.1093/hr/uhac084

Article

## Coordinated transcriptional regulation of the carotenoid biosynthesis contributes to fruit lycopene content in high-lycopene tomato genotypes

James R. Duduit[1,†], Pawel Z. Kosentka[1,†], Morgan A. Miller[1], Barbara Blanco-Ulate[2], Marcello S. Lenucci[3], Dilip R. Panthee[4], Penelope Perkins-Veazie[5] and Wusheng Liu[1,*]

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[5]Department of Horticultural Science, Plants for Human Health Institute, North Carolina State University, North Carolina Research Campus, Kannapolis, NC 28081, USA
*Corresponding author. E-mail: wliu25@ncsu.edu
[†]James R. Duduit and Pawel Z. Kosentka contributed equally.

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- **NC13506** has a good foliage coverage for the protection of fruits
- Fruits are dark-red in color and smooth



- Plants are vigorous and fruits are attractive in color and size.
- Fruits are bigger than Mountain Honey and Mountain Vineyard.


Mountain Rouge

Susceptible Variety

- Pink fruited heirloom-type hybrid tomato variety
- Indeterminate growth habit, vigorous vine
- Late blight resistant (left) is still holding up well whereas susceptibel tomato (right) was completely devastated
- Rated as the best flavored tomato
- Fruit size = 330 gram

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# ‘Mountain Vineyard’ (NC10235) Released



- Indeterminate growth habit with short internode
- Fruit
    - Immature have a glossy finish with dark green shoulders
    - Deep red color from *crimson* gene
    - Firm in the fully ripened stage
    - Long ovate in shape
    - Good resistance to cracking and bursting
- Fruit pedicels are jointless (*j2* gene)
- Flavor and fruit texture rated excellent in subjective taste evaluations in research station plots by growers and consumers




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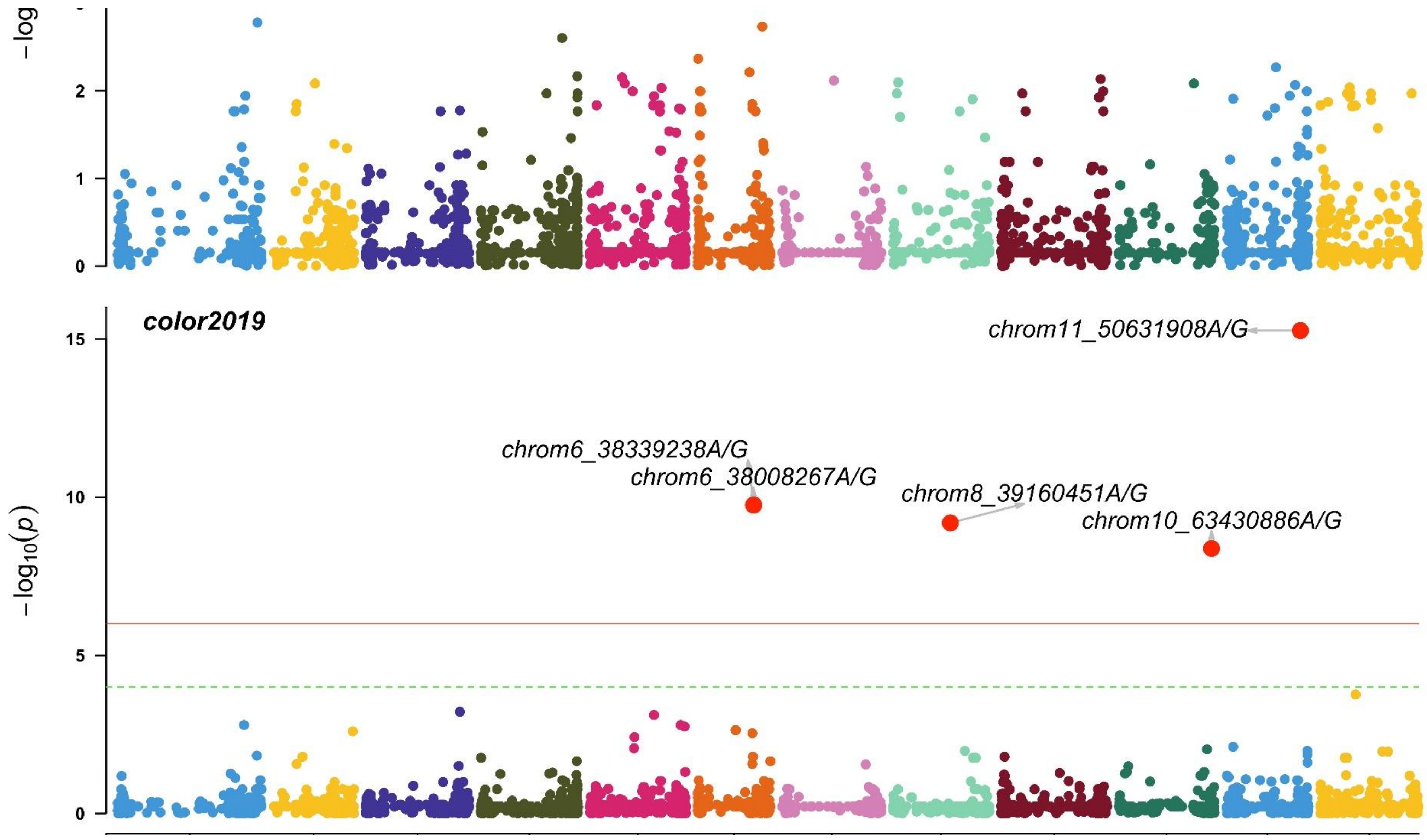
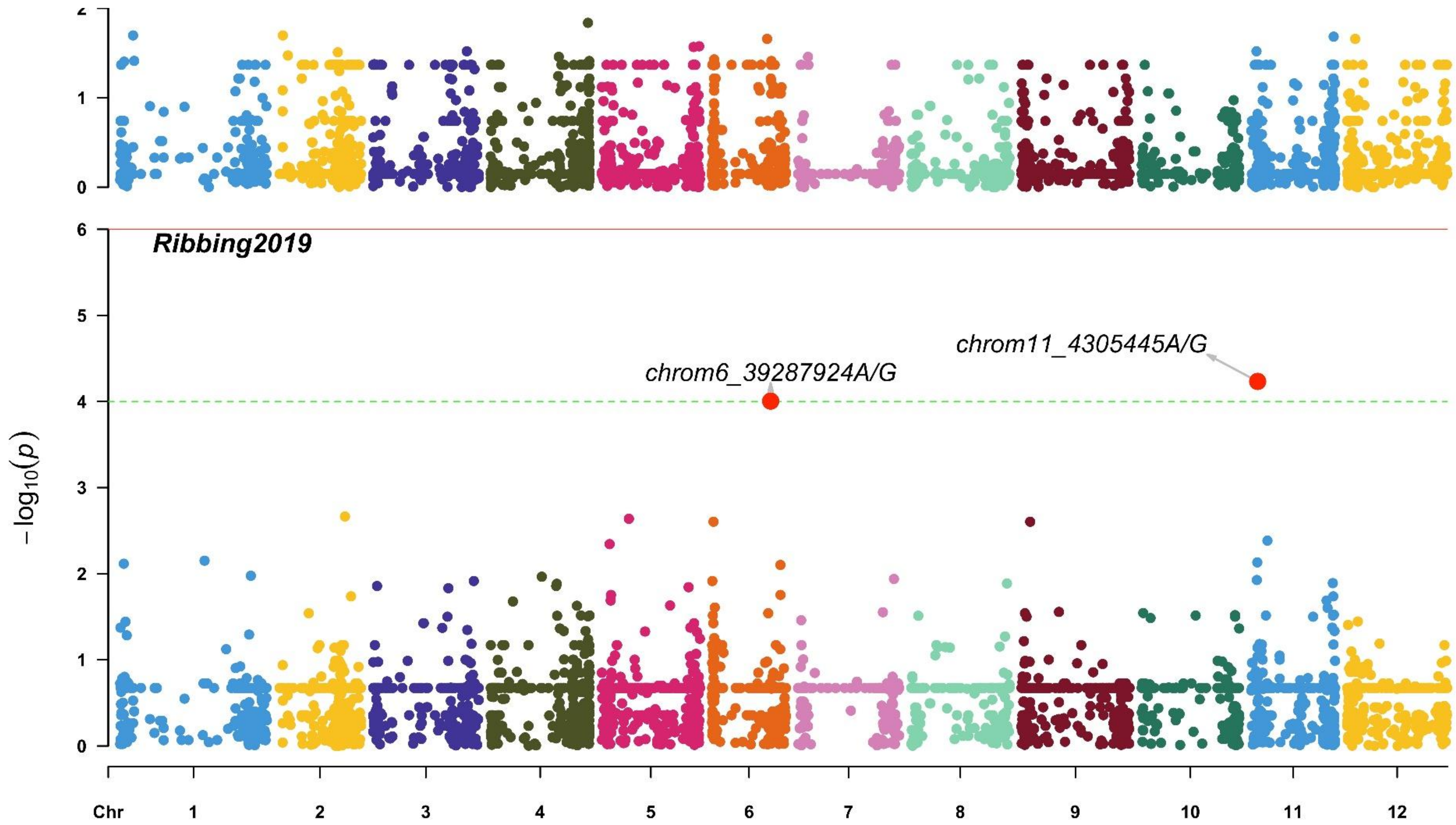
# Released breeding lines and hybrids

| Genotype | Year |
|---|---|
| Mountain Merit | 2010 |
| Mountain Majesty | 2011 |
| Mountain Honey | 2013 |
| Mountain Vineyard | 2013 |
| Mountain Rouge | 2014 |
| Mountain King | 2014 |
| Mountain Lion | 2014 |
| Mountain Bebe | 2018 |
| Mountain Regina | 2018 |
| Mountain Crown | 2018 |

| Genotype | Year | Genotype | Year |
|---|---|---|---|
| NC 714 | 2011 | NC 1 Plum | 2018 |
| NC 4 Grape | 2013 | NC 9 Grape | 2019 |
| NC 5 Grape | 2013 | NC 3 LF | 2019 |
| NC 6 Grape | 2013 | NC 4 LF | 2019 |
| NC 161L | 2014 | NC 7 LF | 2020 |
| NC 706 | 2014 | NC 2 Plum | 2020 |
| NC 7 Grape | 2018 | NC 3 Plum | 2020 |
| NC 8 Grape | 2018 | NC 4 Plum | 2020 |
| NC 1 LF | 2018 | NC 5 Plum | 2020 |
| NC 2 LF | 2018 | | |



−log
color2019
chrom11_50631908A/G
chrom6_38339238A/G
chrom6_38008267A/G
chrom8_39160451A/G
chrom10_63430886A/G
$-\log_{10}(p)$

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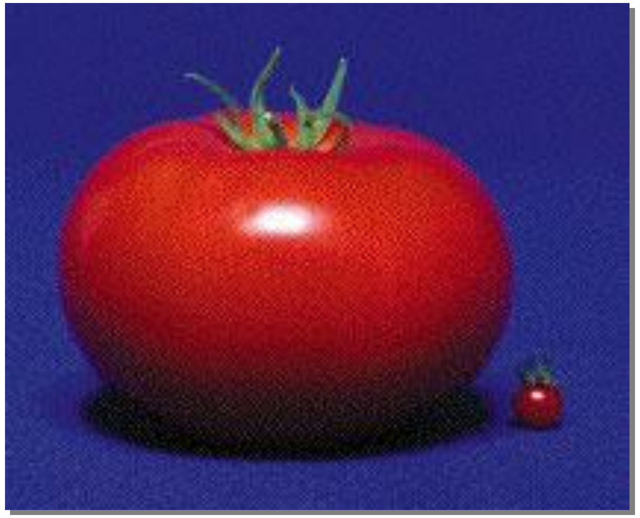
# Improving Our Crops



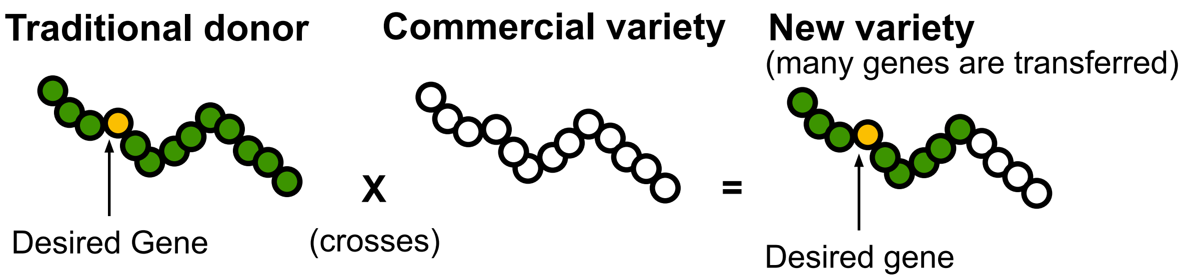
## How are new varieties of crops obtained ?

Domestication
Selection
Breeding
Cloning
Grafting
Hybridization
Mutagenesis

Tissue Culture
Somaclonal Variation
Embryogenesis
Anther Culture
Cell Fusion
Transposons
Genetic engineering

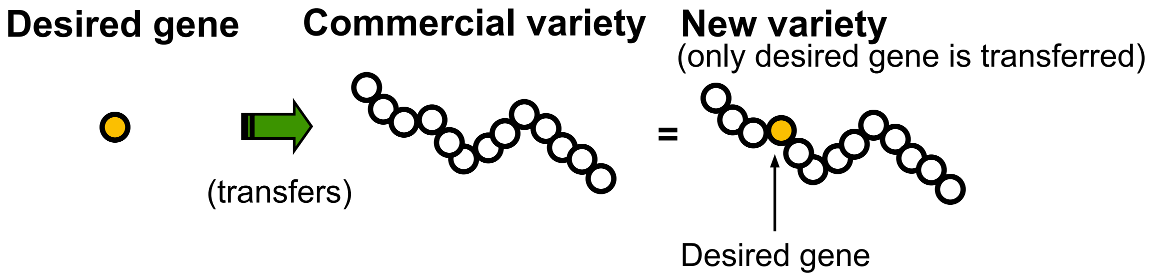
# Traditional Plant Breeding

DNA is a strand of genes, much like a strand of pearls. Traditional plant breeding combines many genes at once.




# Genetic Engineering

Using plant biotechnology, a single gene may be added to the strand.

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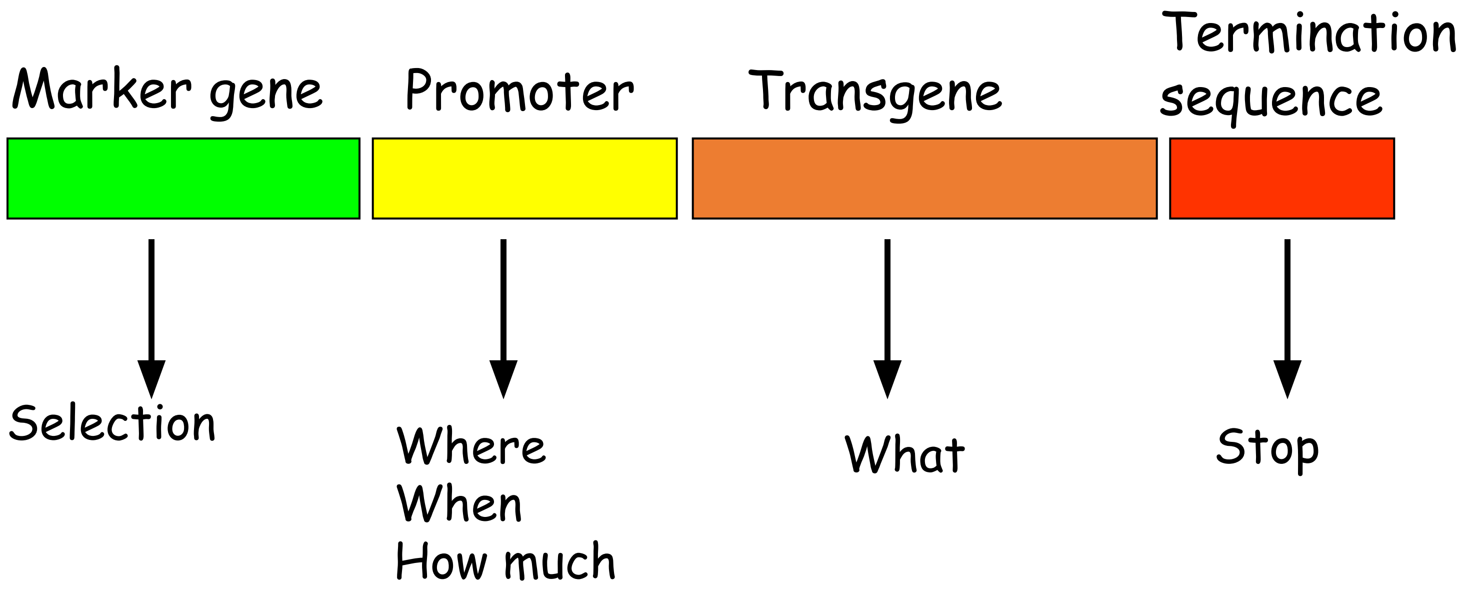
# How is it done?



# Progression of GM plants

- 1st Generation: Input traits (herbicide tolerance, insect resistance, etc.)
- 2nd Generation: Output traits: (pharmaceuticals, enhanced nutrition, etc.)
- 3rd Generation: Non-ag- (phytoremediation, sentinels, detectors)

# Examples of Current and Future Application

## Insect resistance genes

Cotton

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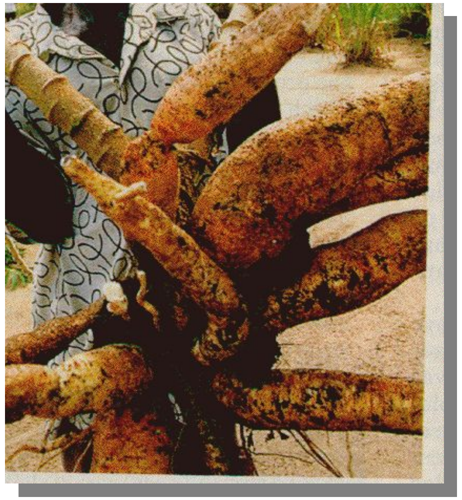
Corn



# Cassava

- Eaten by 500 million Africans
- Very productive, drought-tolerant

Healthy Cassava

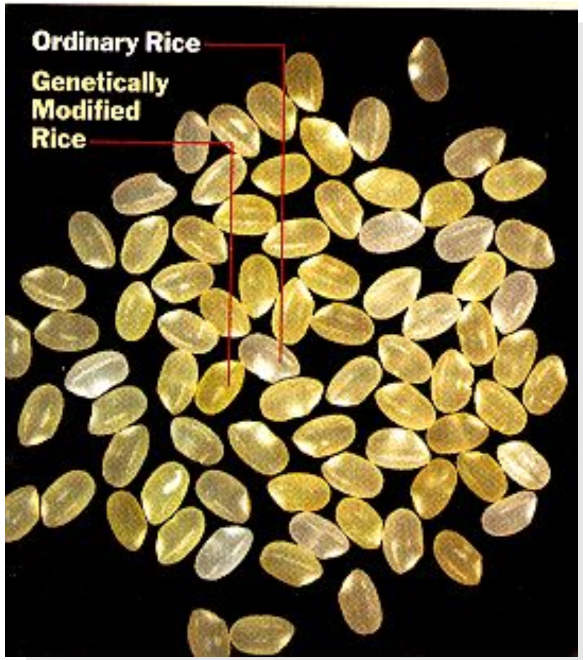
African Cassava Mosaic Virus-infected Cassava

# Environmental benefits

No-till agriculture– less soil erosion.

Over 1 million gallons of unsprayed insecticide per year.

# Golden Rice




- Milled rice has no beta-carotene
- Vitamin A deficiency - 200 million children and woman
- About 500,000 children go blind (60 every hour!)
- 2 million children die each year
- Golden Rice may provide one of the many solutions


Ingo Potrykus (Switzerland)
and Peter Beyer (Germany)

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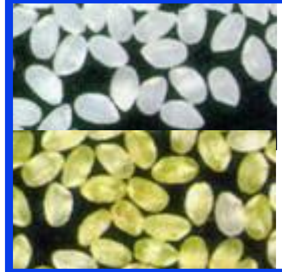
# Health benefits

## High Oleic Soybeans

| | Oleic Acid (% Oil content) |
|---|---|
| Non-Transgenic | 15% |
| Transgenic | 84% |

## High Vitamin E Canola

| | Vitamin E (ng/seed) |
|---|---|
| Non-Transgenic | 4 |
| Transgenic | 332 |



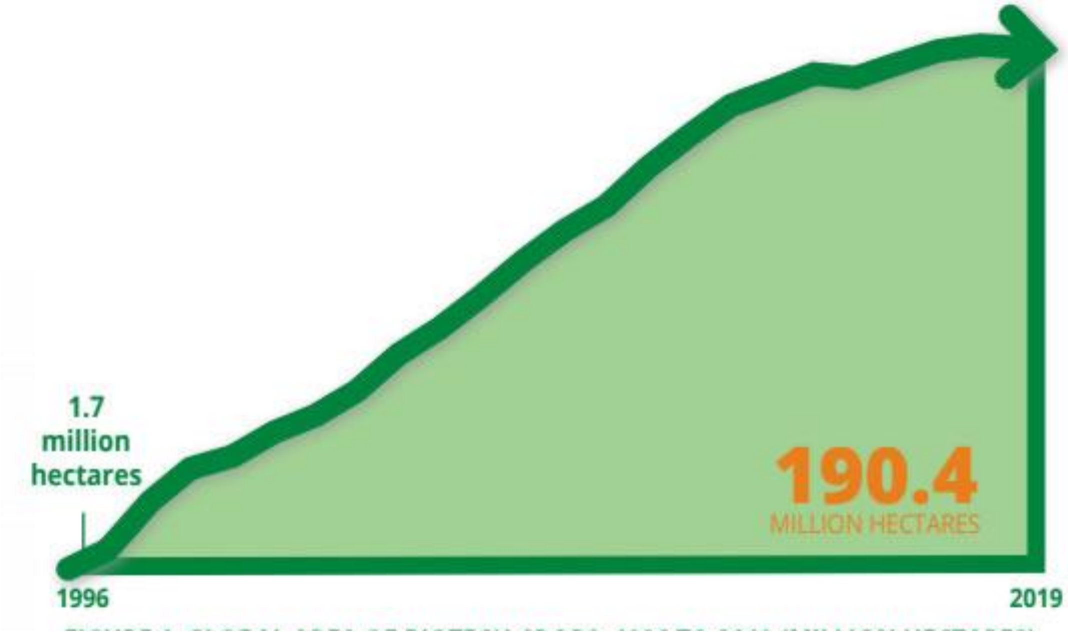
## High Vitamin A Rice

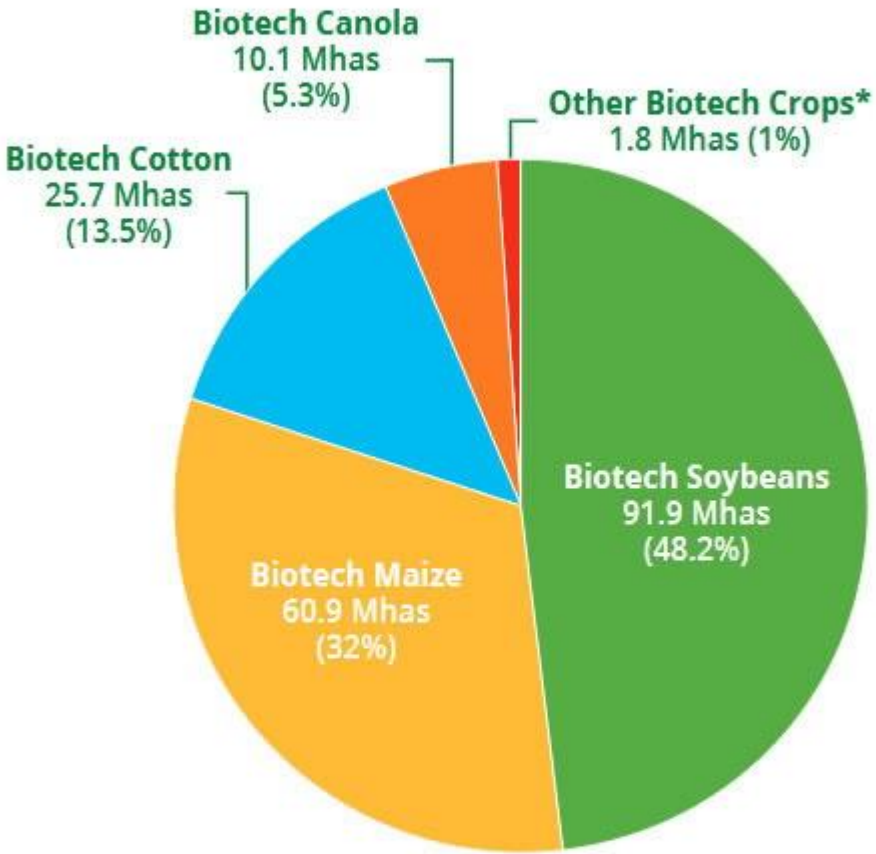
| | Provitamin A (mg/g carotenoid) |
|---|---|
| Non-Transgenic | 0 |
| Transgenic | 1.6 |

## More Available Iron in Rice

| | Iron (ng/grain) |
|---|---|
| Non-Transgenic | 1403 |
| Transgenic | 3810 |

# Statistics of biotech crops in the world



# Genomic database


https://www.isaaa.org/resources/publications/pocketk/16/

https://subjectguides.lib.neu.edu/bioinformatics/databases

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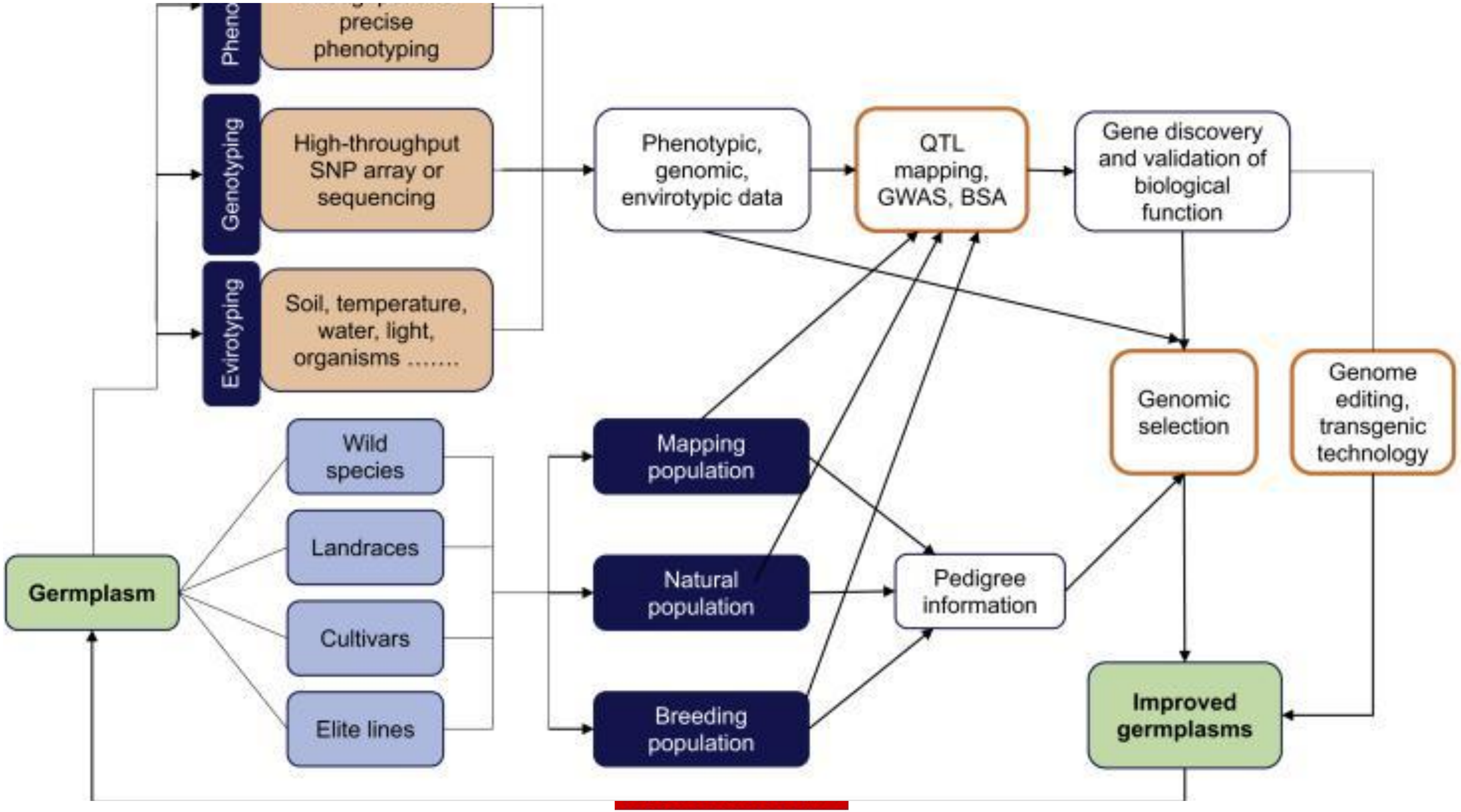
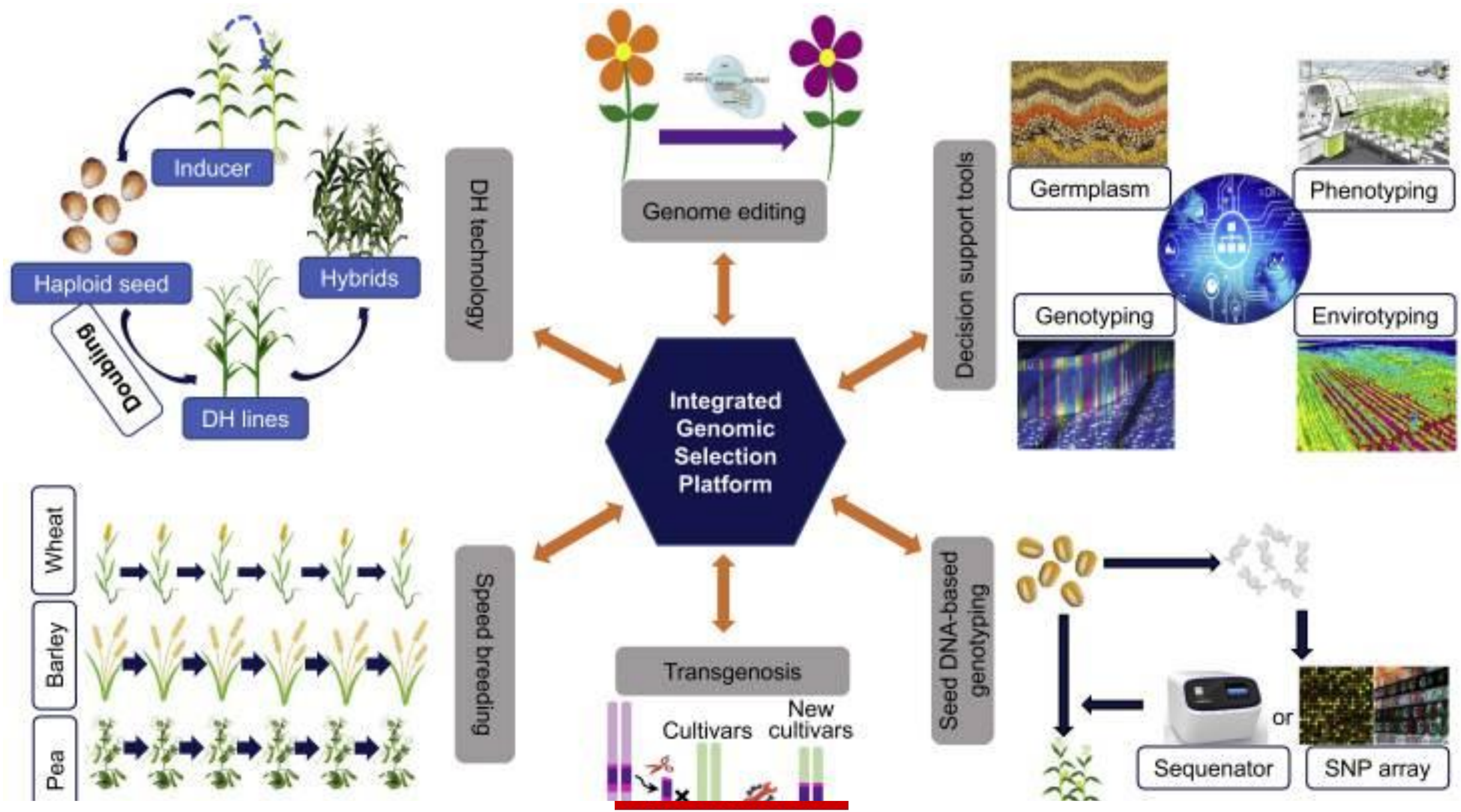
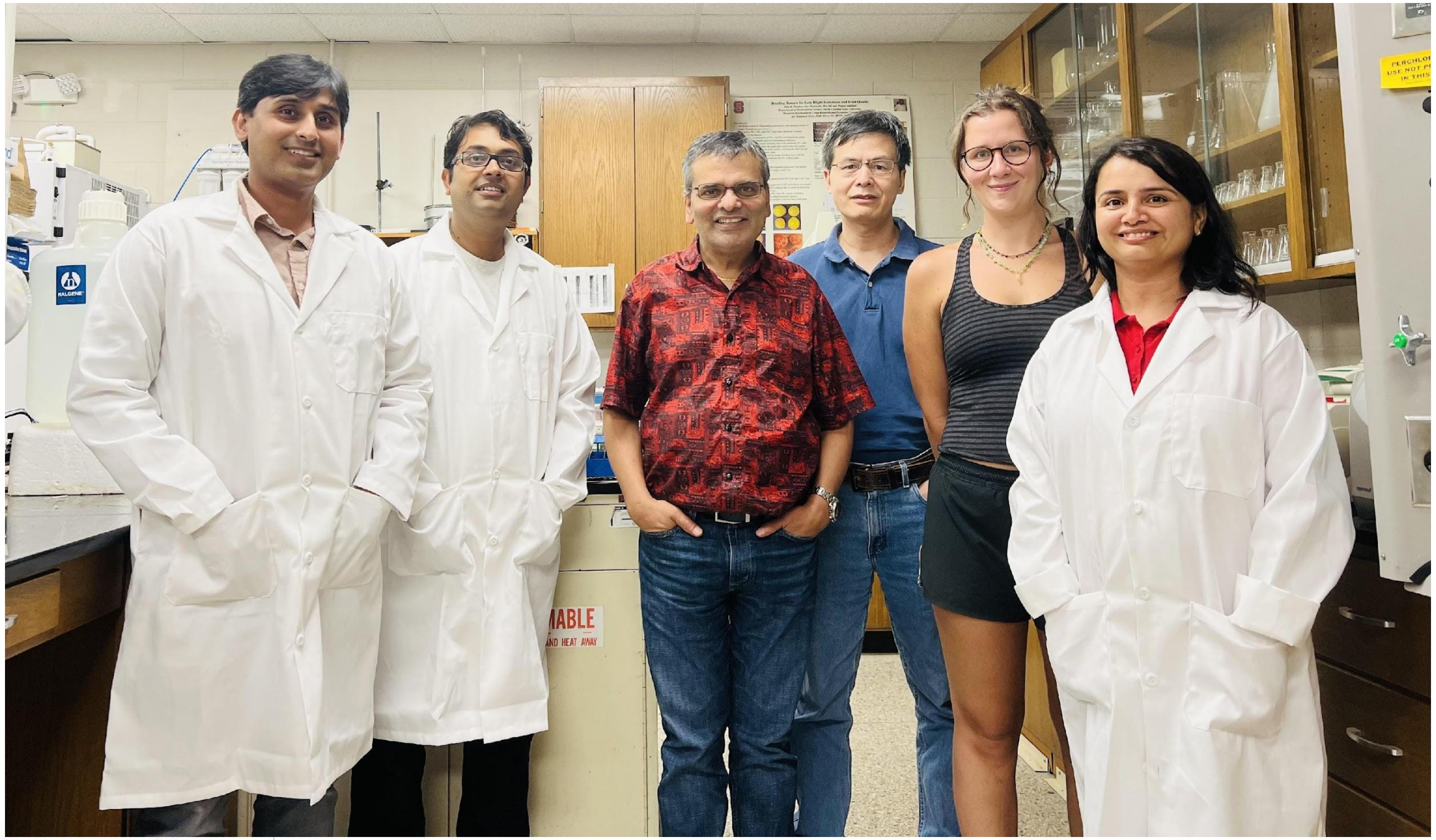


precise phenotyping
Pheno
Genotyping
High-throughput SNP array or sequencing
Evirotyping
Soil, temperature, water, light, organisms .......
Phenotypic, genomic, envirotypic data
QTL mapping, GWAS, BSA
Gene discovery and validation of biological function
Genomic selection
Genome editing, transgenic technology
Wild species
Landraces
Cultivars
Elite lines
Germplasm
Mapping population
Natural population
Breeding population
Pedigree information
Improved germplasms


Inducer
Haploid seed
Doubling
DH lines
Hybrids
DH technology
Genome editing
Decision support tools
Germplasm
Phenotyping
Genotyping
Envirotyping
Integrated Genomic Selection Platform
Speed breeding
Wheat
Barley
Pea
Transgenosis
Cultivars
New cultivars
Seed DNA-based genotyping
or
Sequenator
SNP array

# Acknowledgements

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NSF

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Questions?