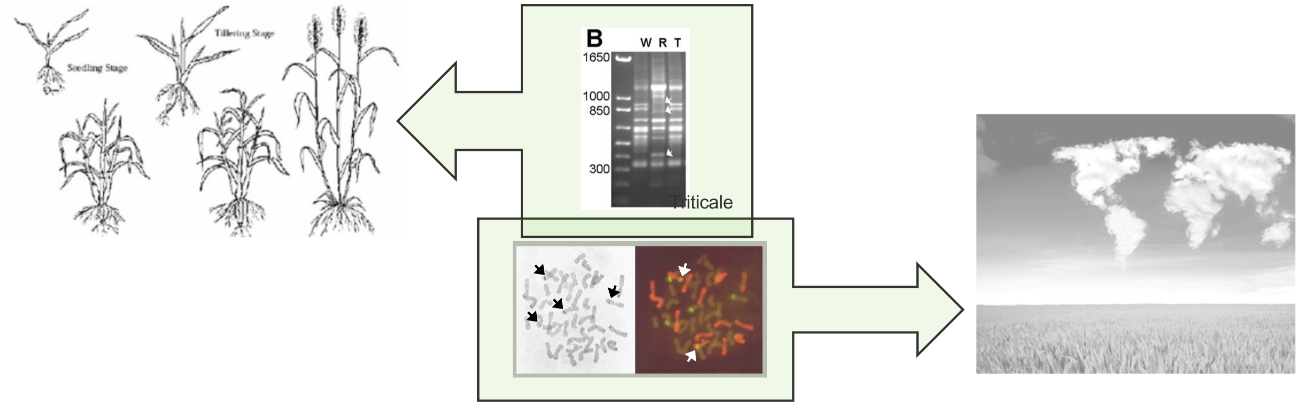
Mestrado em Engenharia Alimentar

Tecnologia dos Cereais
Cereal Evolution and Genomics

Manuela Gomes da Silva



Índice

Understanding **Cereal Genomics**

Cereals economic and scientific **importance**

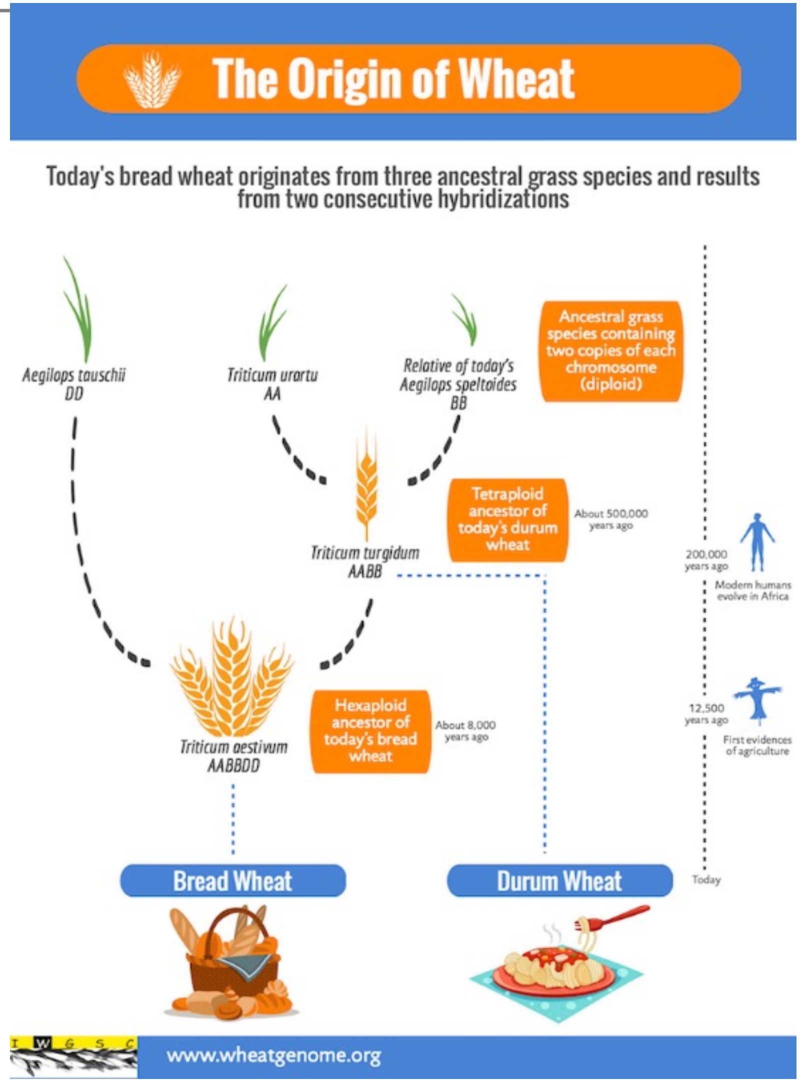
→ extensive history of research in **genetics, development**, and **evolution**

Relationships among morphologically diverse cereals from globally geographic environments

→ particularly attractive for comparative studies of **plant genome evolution**

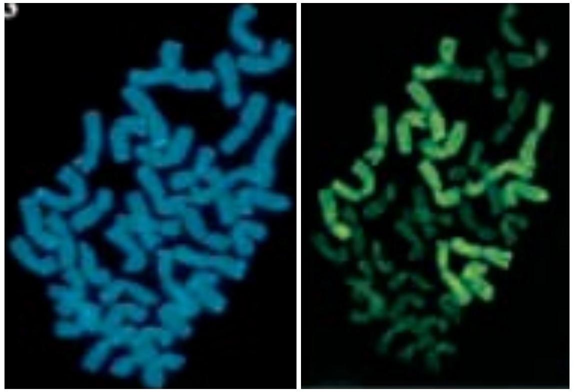
Complete genome sequence of rice

→ transition to high-throughput **genomics study** of many other cereals



International **Wheat** Genome
Sequencing Consortium **IWGSC**

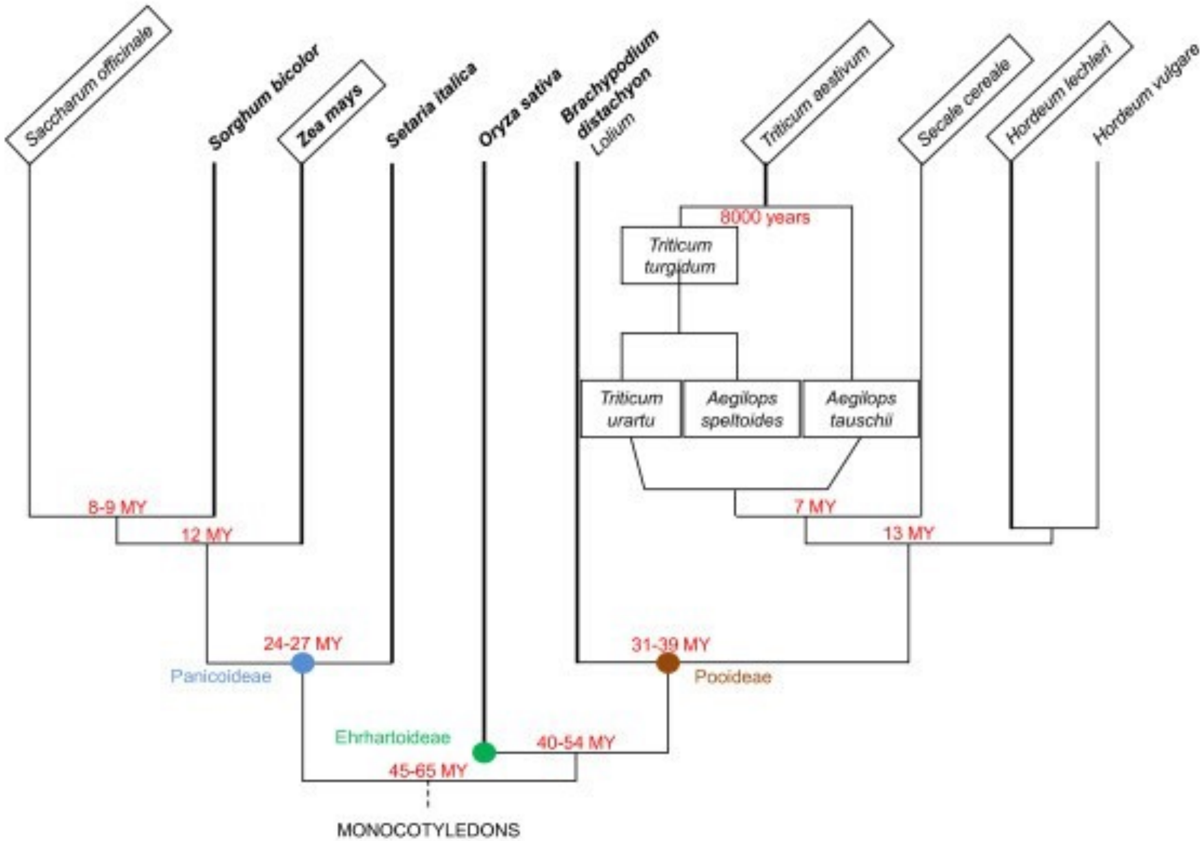
Importance of cytogenetics and molecular biology for cereal study



FISH of hexaploid triticale metaphase

Need to **know** and understand the evolution of **cereal genomes**

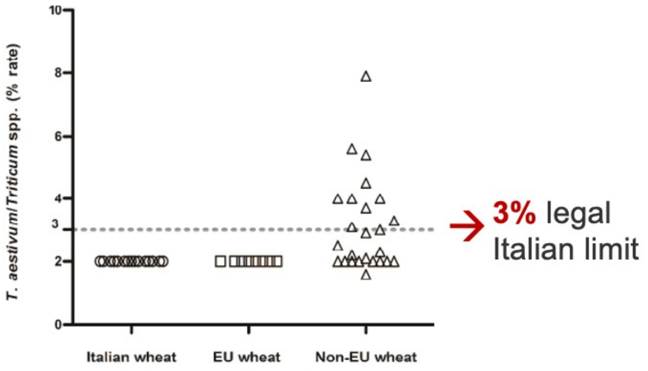
Design strategies for **plant breeding**



Poaceae phylogenomics based on a biosynthetic pathway

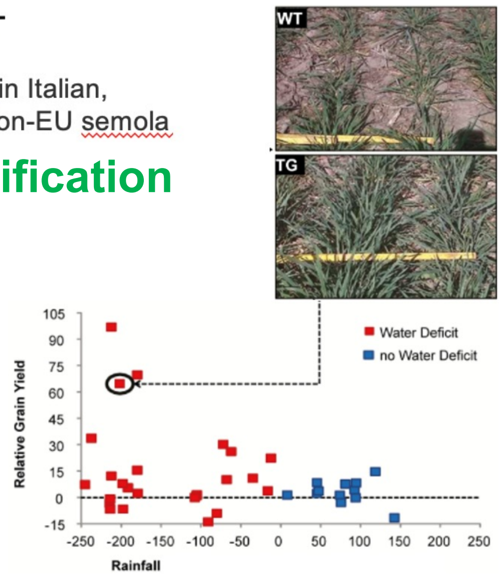
Dutartre et al 2012

Use of **Molecular Biology** tools

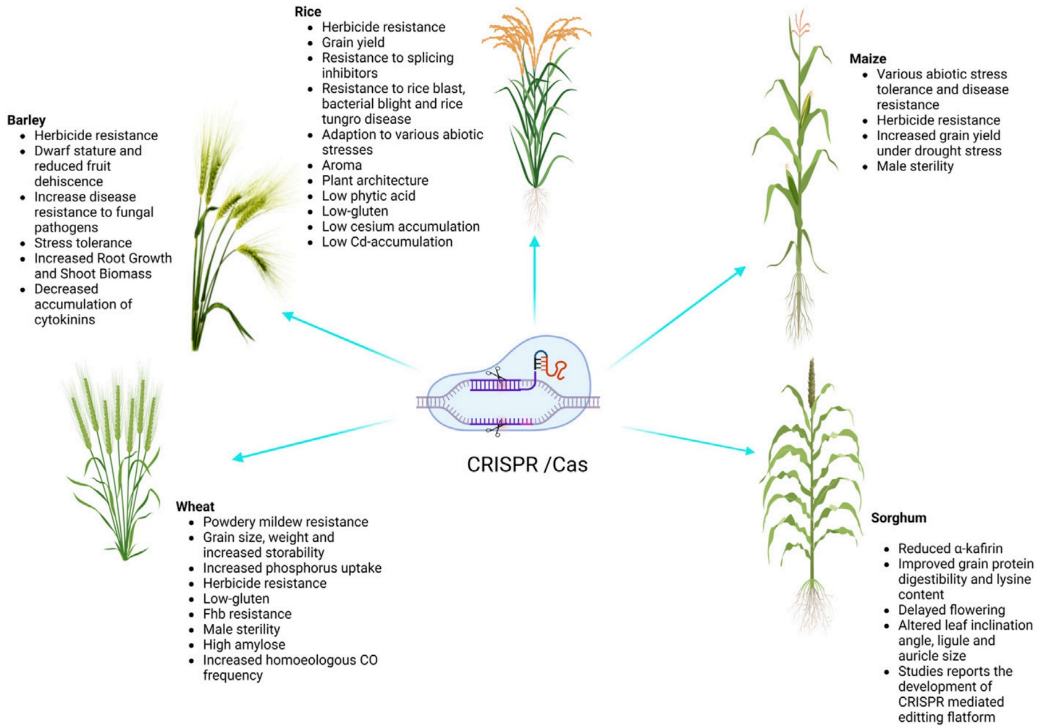


Quantification of *T. aestivum* in Italian, European Union (EU), and Non-EU semola

Italian pasta certification



OGM wheat drought tolerance



Successful applications of CRISPR/Cas

Importance of **Cereal Biotechnology**

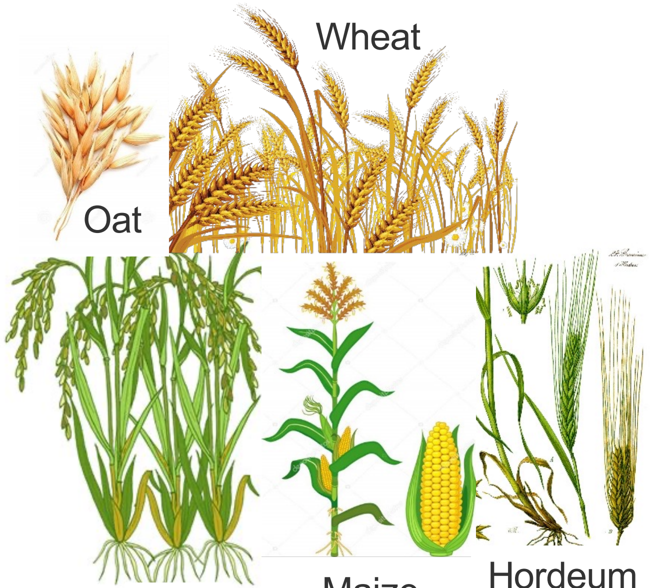
Polyploid plants - **Gramineae**

Many crop plants are polyploid

Potato, cotton, coffee, tobacco, banana…

A sample of agricultural products obtained from polyploid crops

Polyploidy is particularly frequent in Poaceae family (**Gramineae**)

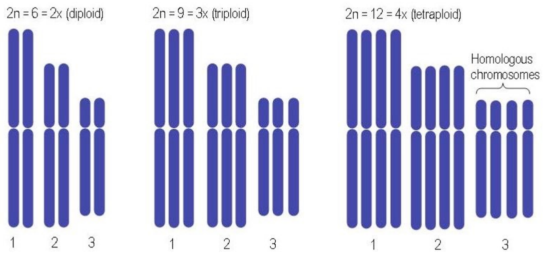


Leitch and Leitch, 2008

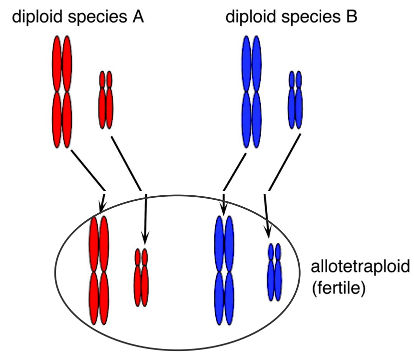
Polyploid types

Multiple genomes → organisms with multiple copies of a genome or with different genomes sharing the same nuclei (Stebbins, 1971, Lewis, 1980)

- **Auto**polyploid ← duplication of one genome
  (somatic chromosome duplication
  or union of unreduced gametes)



- **Allo**polyploid ← combination of 2 or more different genomes
  (hybridization + somatic chromosome duplication
  or union of unreduced gametes)

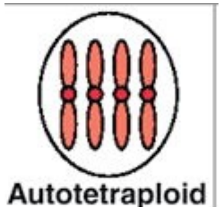


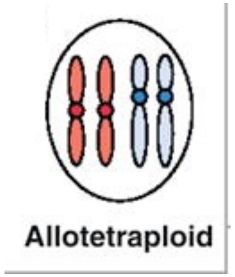
Allopolyploids considered to be much **more common** than autopolyploids.

Levy and Feldman, 2002

Polyploidy

- POLYPLOID - plants with multiple copies of a genome or with different genomes
  - Autopolyploid ← **duplication** of one genome
  - Allopolyploid ← **combination** of 2 or more different genomes

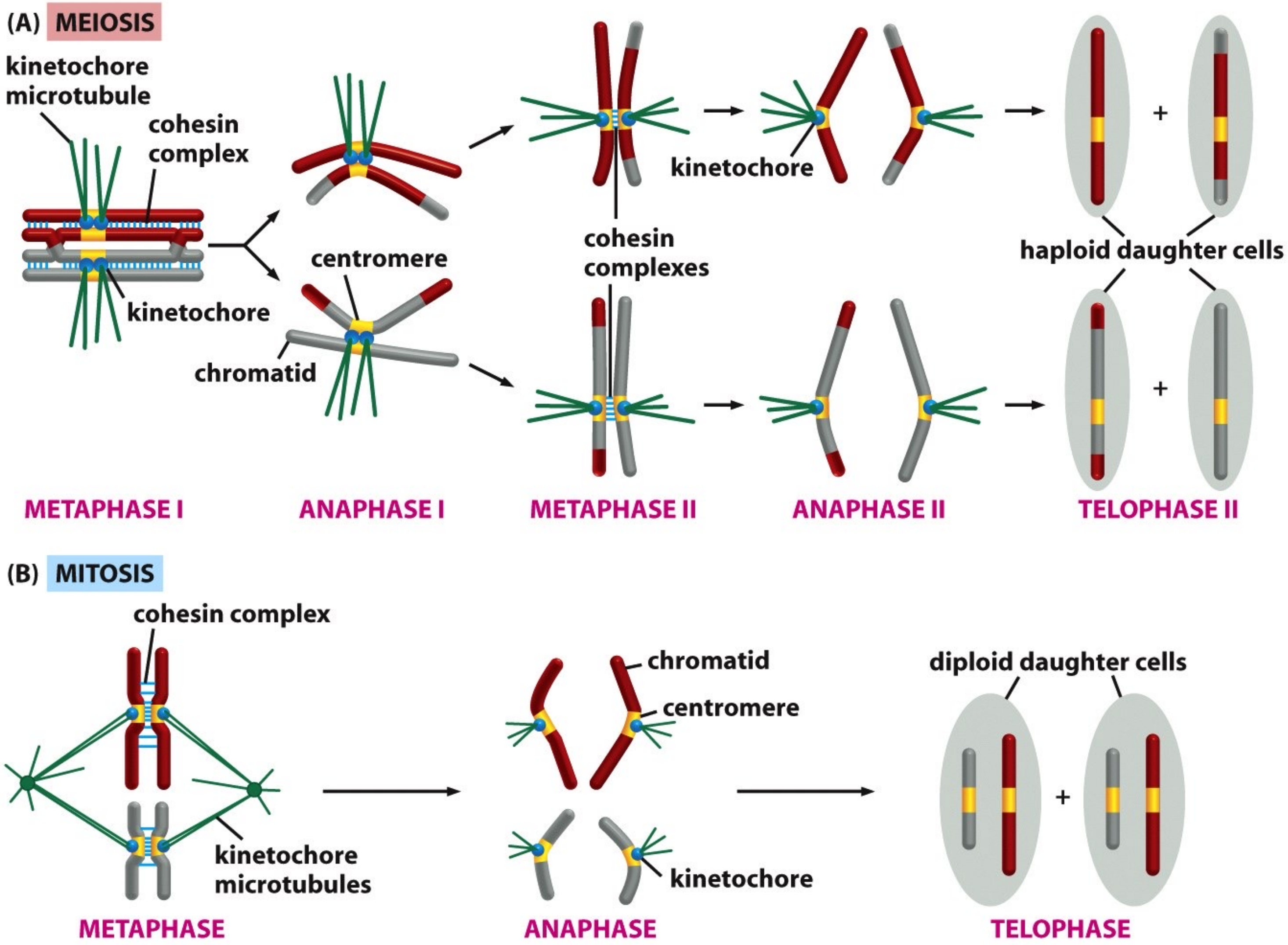




How do polyploid plants arise??

→ **Mitotic or Meiotic errors**

Rare events that occurred at least one time throughout the evolutive history



1 duplicação - 2 divisões
→ 4 células haploides (n)

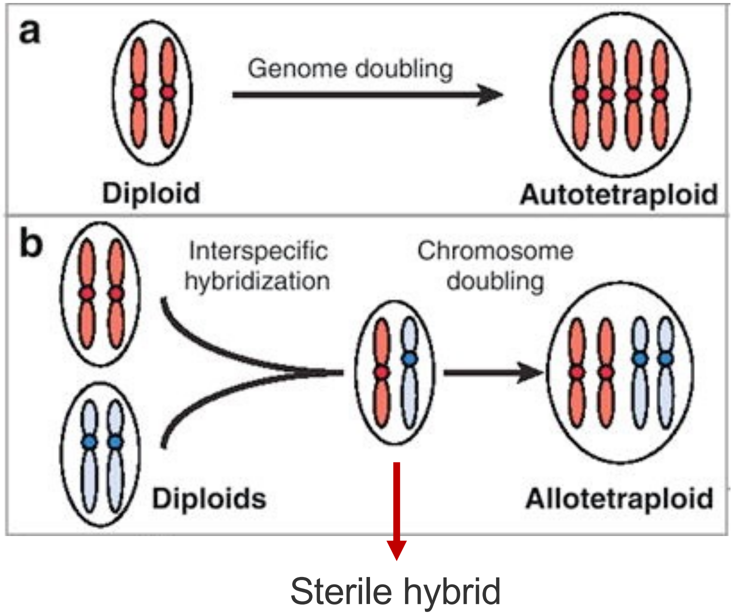
1 duplicação - 1 divisão
→ 2 células diploides (2n)

Polyploidy origin - **Mitotic** anomalies

→ **somatic chromosome doubling**

Diploid species doubling → **autopolyploid**

F1 hybrid doubling → **allopolyploid**

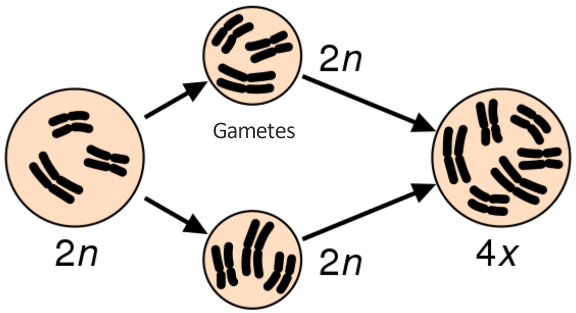
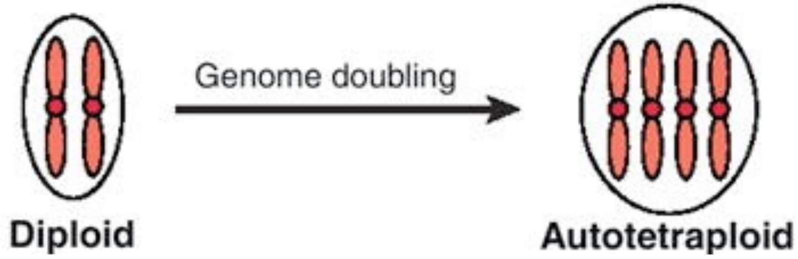


(a) Formation of an **autotetraploid** by doubling a basic set of chromosomes.

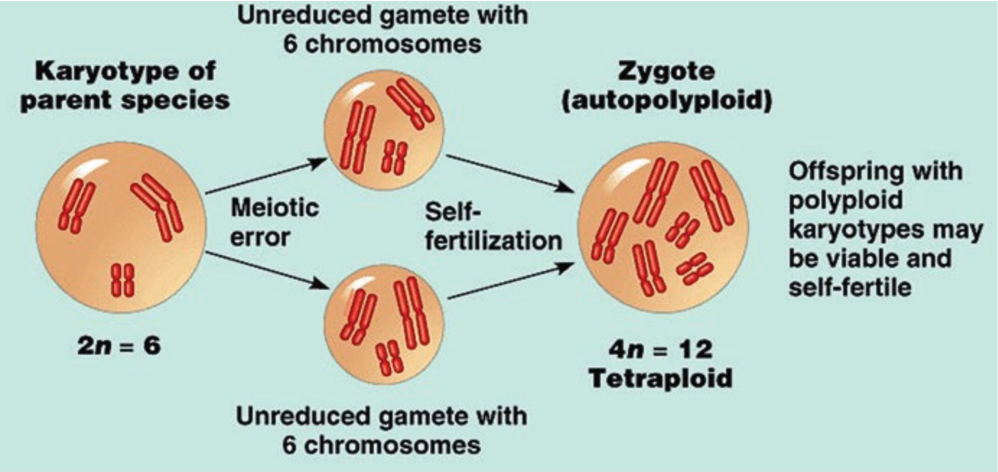
(b) Formation of an **allotetraploid** by **interspecific hybridization** followed by **chromosome doubling**.

Autopolyploidy ← genome duplication

Causes of **genome duplication**:

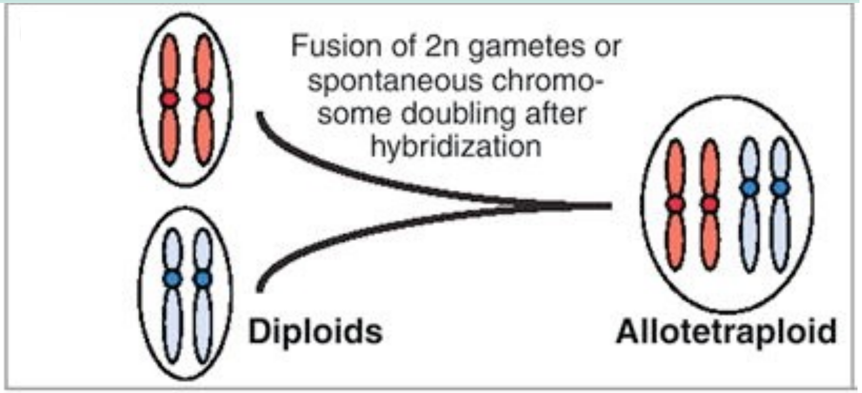
Meiotic non-reduction of gametes (both in egg and sperm)	
DNA replication **without mitotic** division	

Polyploidy origin - Fusion of diploid gametes



Fusion of unreduced gametes from a diploid species.

→ **Autopolyploid**



Fusion of unreduced gametes in two different diploid species.

→ **Allopolyploid**

Chen, 2007

Formation of **allopolyploids**: **main models**

"Two-step" model

interspecific hybridization

chromosome doubling of the F1 hybrid

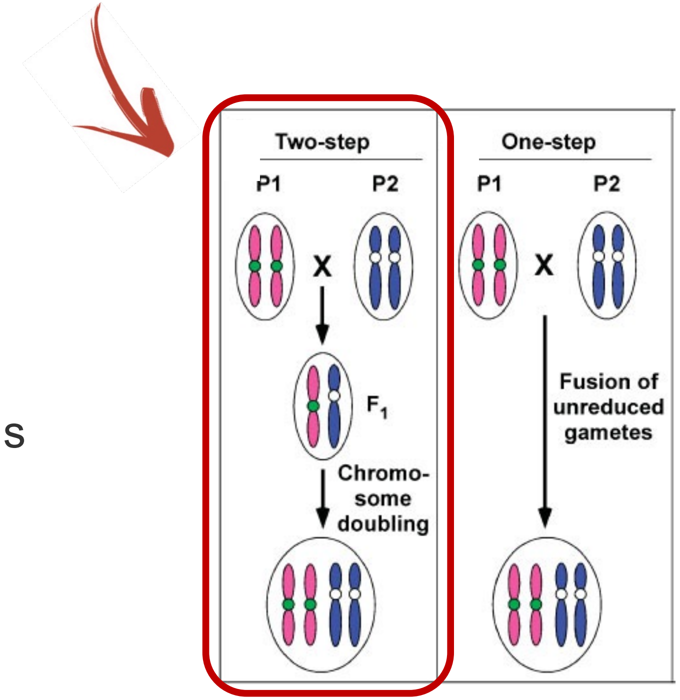
→ most common process

- in **Triticeae** evolution
- to produce **synthetic** polyploids

(colchicine chromosome-doubling properties)

"One-step" model

fertilization of unreduced gametes from different diploid species



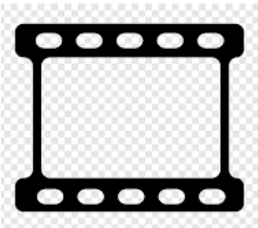
Chen, 2007

Actividade – visualização de vídeos em grupo

- Grupos de 4/5 alunos
- Cada grupo vê um **vídeo** (10 min)
- Cada grupo discute o conteúdo do vídeo e elabora um pequeno **resumo** (10 min)
- Cada grupo elege um porta-voz
- No fim, **apresentação** do resumo sobre cada um dos vídeos (5 min)

VÍDEOS

https://tinyurl.com/VideosAulaTecCereais



1 - Mitosis vs. Meiosis: Side by Side Comparison 6’21’’

https://www.youtube.com/watch?v=zrKdz93WlVk&ab_channel=AmoebaSisters

2 - Polyploidy 2’

https://www.youtube.com/watch?v=e77Dwu7-QMo&ab_channel=BaylorTutoringCenter

3 - Sweet but dangerous? The strange story of polyploidy 4’11’’

https://www.youtube.com/watch?v=Idpt19_PSVQ&ab_channel=GatsbyPlantScienceEducationProgramme

4 - FISH - Fluorescence In Situ Hybridization 3’48’’

https://www.youtube.com/watch?v=LiRJoTi44TA&ab_channel=Henrik%27sLab

5 - Sanger Sequencing of DNA 3’39’’

https://www.youtube.com/watch?v=AI4CnG5Jp4s&ab_channel=LaUrsa

Vídeo

Cada grupo vê um **vídeo** (10 min)

10:00

Vídeo

Cada grupo vê um **vídeo** (10 min)

10:00

Resumo

Discussão e elaboração do **resumo** (10 min)

10:00

Resumo

Discussão e elaboração do **resumo** (10 min)

10:00

Apresentação

Apresentações à turma dos resumos dos vários grupos (5 min)

05:00

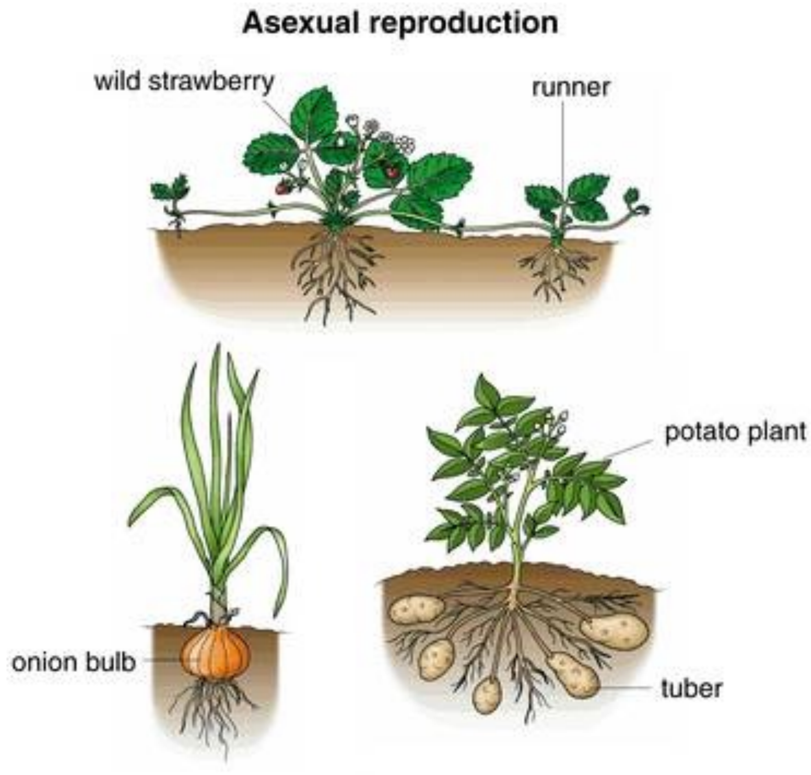
What **plants** do better than animals?

In terms of reproduction, plants have more options than animals:
→ sexual and asexual

- Hybridization
  - Vegetative reproduction
- Polyploidy

Species A X Species B
↓
Sterile hybrid

Animals → Usually the end!
Plants → Vegetative reproduction (Polyploidy)



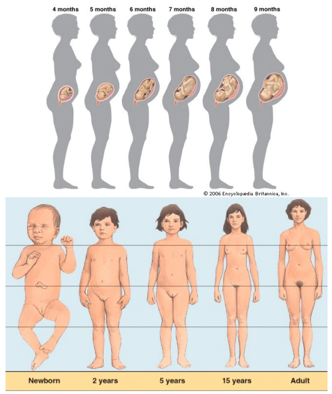
Why Polyploidy is less common in **animals**?

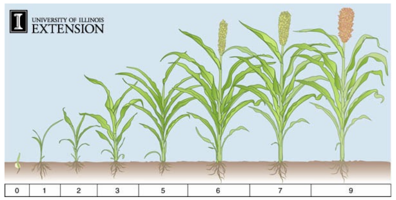
ANIMALS

Chromosomally determined **sex** (polyploidy interference)

More complex **development** - organ systems fine-tuned affected by different gene dosages

Isolation mechanisms (geographic, temporal, behavioral etc.) prevent natural interbreeding between species





PLANTS

Meristematic tissue throughout their lives and self-fertile

Advantages of Polyploidy

- **Duplicated gene copies can evolve** to assume new functions

  → **Genes free to mutate**

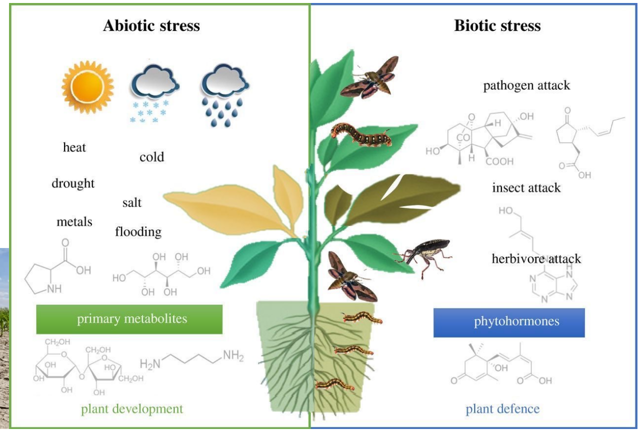
- **Larger** cells and organs

- Enhanced abiotic and biotic **stress tolerance**

- Recurrent polyploidy (increased genetic diversity)
- Genomic rearrangements (novel genotypes**)**
- Epigenetic changes (rapid adaptation)

→ Higher **PLASTICITY**

Increased **adaptability** to a higher ecological range



Polyploidy → Larger cells and organs

Diploid and autotetraploid cultivars of

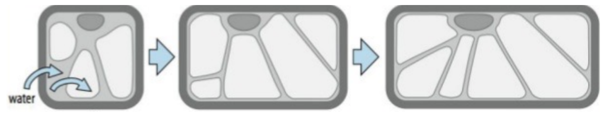
Lolium perenne and *L. multiflorum*

- **longer leaves**, longer mature cells (20x) (epidermal and mesophyll)

  ← **faster cell elongation rate**,

  not by a longer period of cell elongation

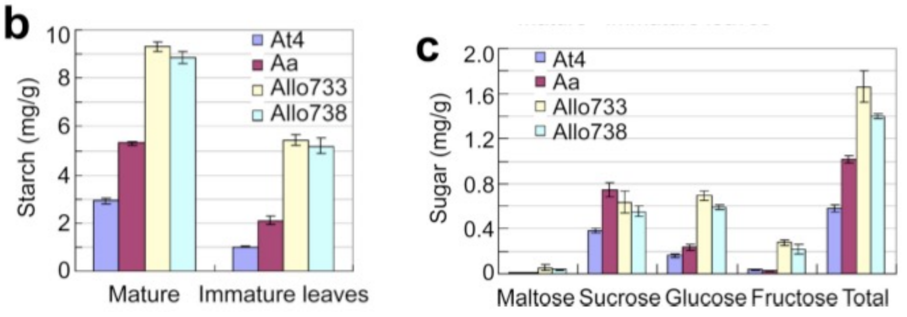
- No variation in cell division parameters (cell production rate and cell cycle time)



Sugiyama , 2005

Molecular basis for **polyploid vigor**

Allopolyploid → genes for photosynthesis and starch metabolism are **more active**



- Increased **photosynthesis**, higher amounts of **chlorophyll**
- higher **starch** accumulation

  → **growing larger** in comparison with their parents

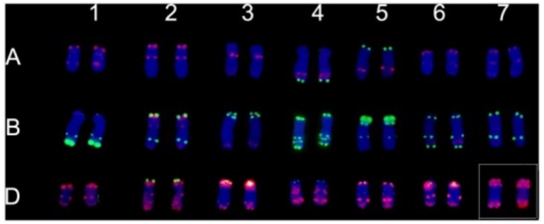
The hybrid *Arabidopsis* plant (center) is larger than its parents (top and bottom), an example of hybrid vigor

Ni et al, 2009

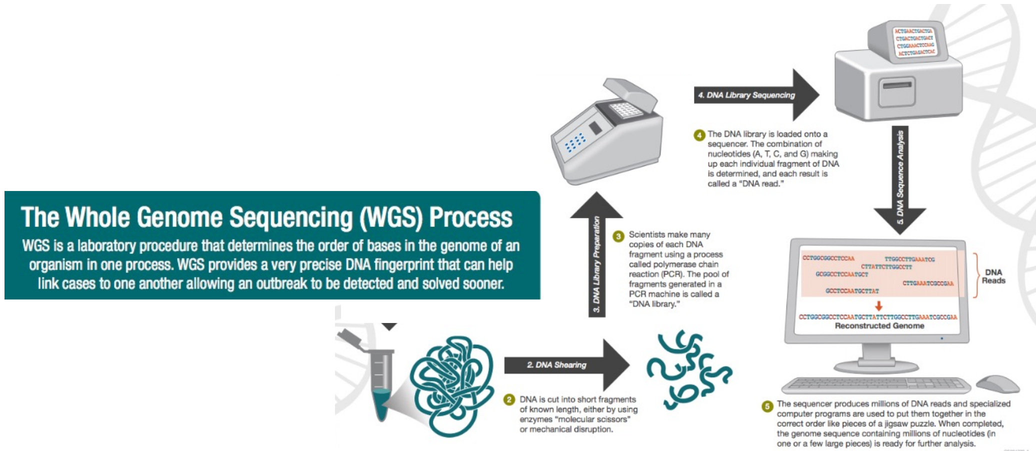
Polyploidy detection

- **Polyploid** species – detected by cytogenetics

Wheat FISH with pTa535 (red) and pSc119.2 (green), chromosomes counterstained with DAPI (blue).



- **Paleopolyploids** – detected by genome sequencing



Cereal genome **cytogenetics**

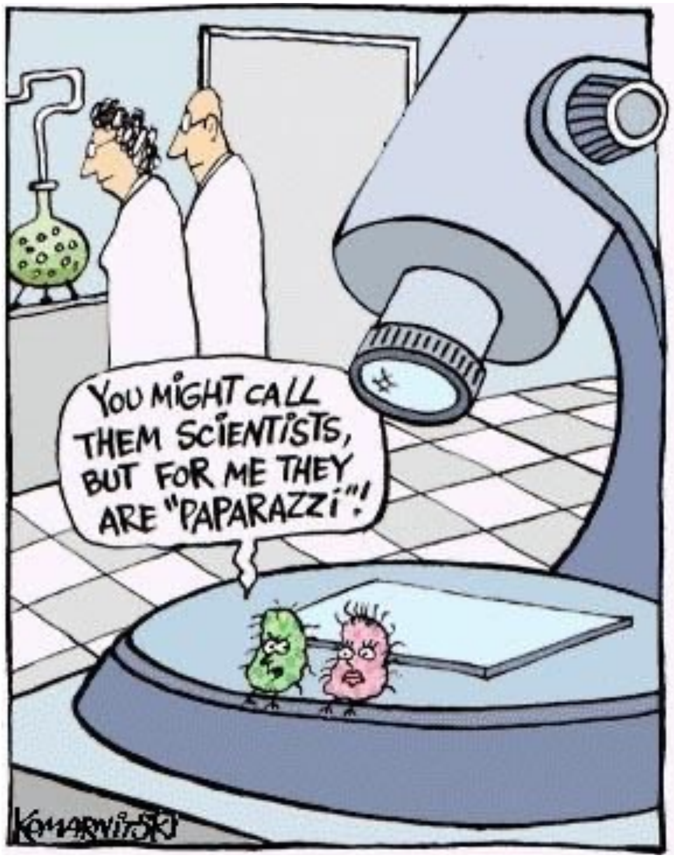
Metodologias citogenéticas

- Estabelecimento de **cariótipos**
- **Técnicas de bandeamento**
  → Actividade
- Caraterização do **genoma dos trigo e espécies aparentadas**

Cytogenetics

Cytogenetics = Cellular Genetics

- Branch of genetics that is concerned primarily in **cellular components**, especially **chromosomes**
- Correlates the structure and number of chromosomes analyzed in isolated cells with variation in genotype and phenotype.



Cytogenetic tools

→ Conventional **karyotyping**

Chromosome banding

→ **Molecular cytogenetics**

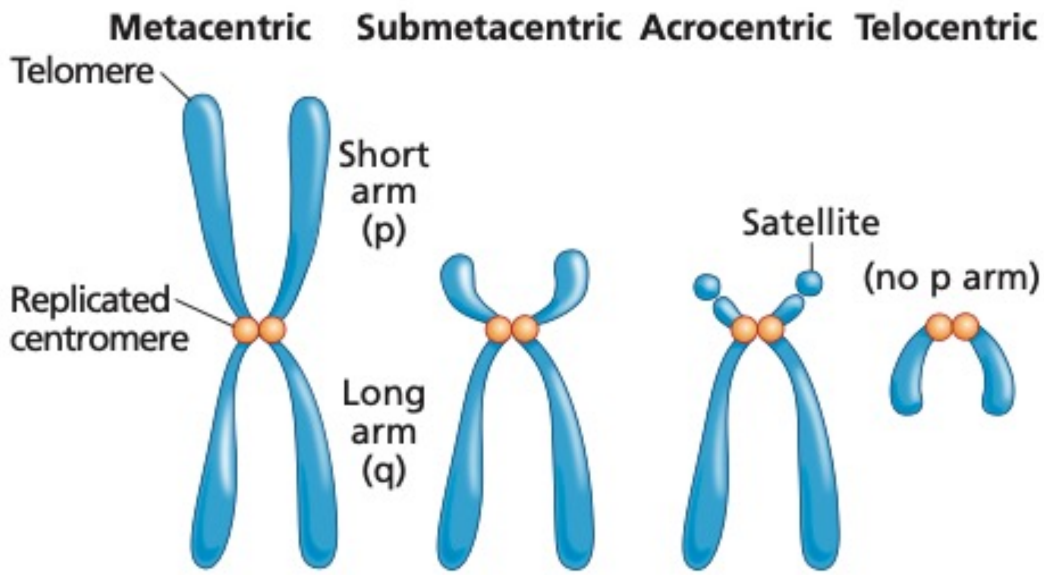
Fluorescence In Situ Hybridization (FISH)

Chromosome morphology

Contromeric position → chromosome form

The form of chromosomes is based on the position of the centromere

Four broad categories are used →

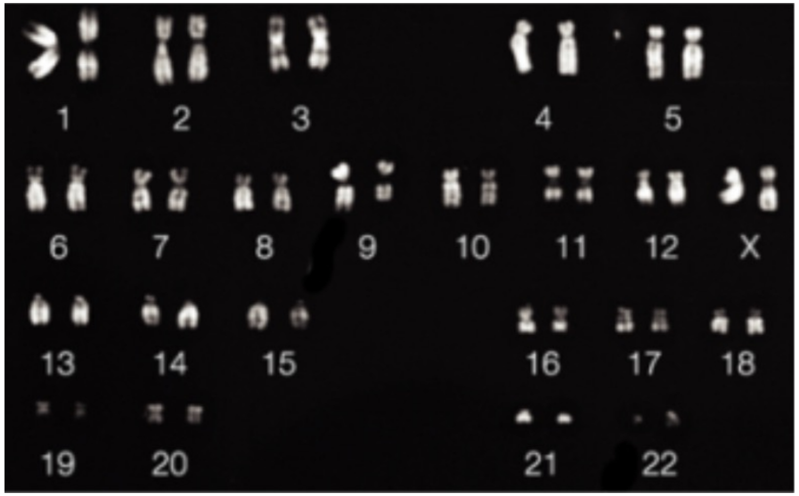


Karyotypes:
Autosomes (non-sex) chromosomes are numbered in **descending order by size.**
Sex chromosomes are generally placed at the **end.**

Chromosome banding Techniques

G-banding

Q-banding



C-banding

Technique	Procedure	Banding pattern
G-banding	Mild proteolysis with trypsine followed by staining with Giemsa	Dark bands are AT-rich Pale bands are GC-rich. Typically, Giemsa staining produces between 400 and 800 bands distributed among the 23 pairs of human chromosomes
R-banding	Heat denaturation followed by staining with Giemsa	Reverse banding: Dark bands are GC-rich Pale bands are AT-rich
Q-banding	Stain with quinacrine	Fluorescence banding. Dark bands are AT-rich Pale bands are GC-rich
C-banding	Denature with barium hydroxide and then stain with Giemsa	Dark bands contain constitutive heterochromatin. In humans mainly stains the centromeres

Actividade - Karyotyping exercise

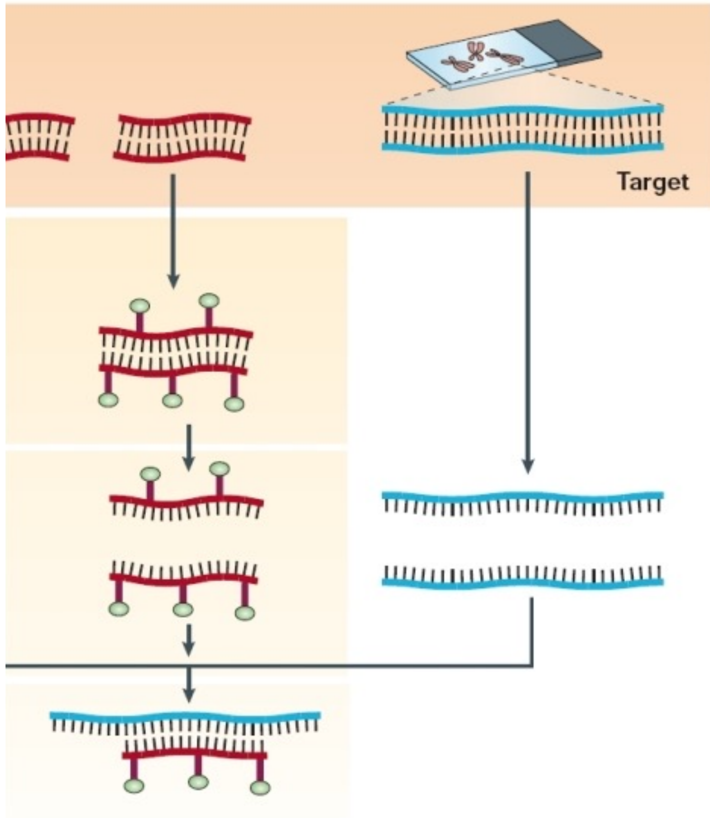
https://ilias.hhu.de/ilias.php?baseClass=ilSAHSPresentationGUI&ref_id=884328

Fluorescence In Situ Hybridization (FISH)
Fundamentos da técnica

- Utilização de **sequências conhecidas (sondas)** marcada com um **fluorocromo**
- **Desnaturação** da **sonda** e das **sequências** de DNA nos **cromossomas**
- Renaturação (**Hibridação**) da sonda com a sequência nos cromossomas com base na

    Complementaridade entre bases

    → associação da sonda / sequência-alvo

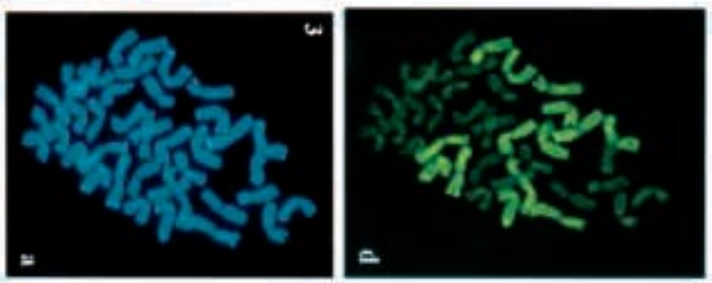


→ visualização de sequências de DNA marcadas nas **células**

In Situ Hybridization
Aplicações da técnica

- **bandeamento** para estabelecimento de cariótipos - **FISH**

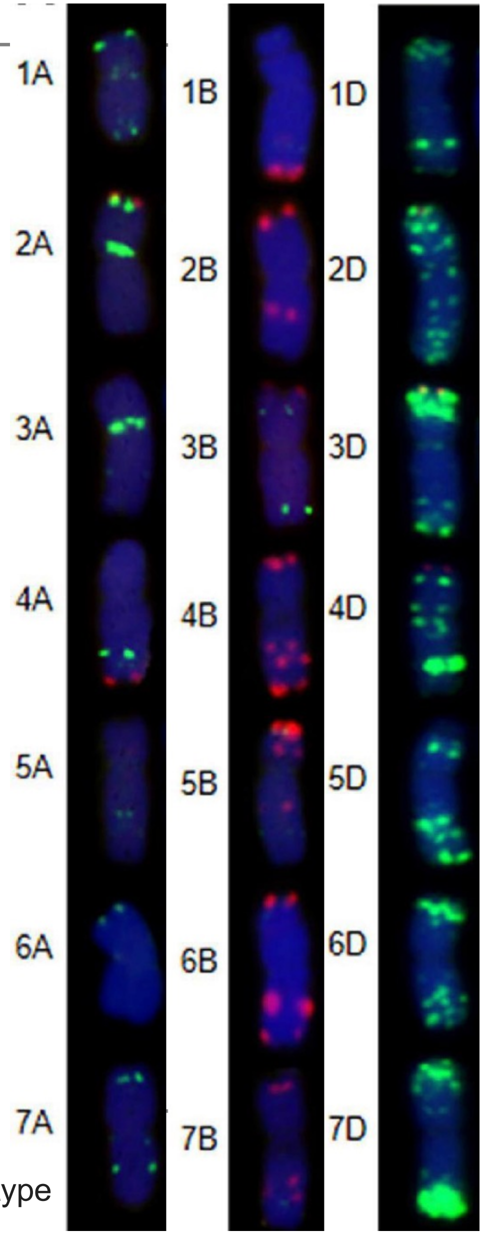
- Identificação de **genomas das espécies parentais** - **GISH**



Cromossomas de triticale

Verde - centeio

Azul – DNA corado com DAPI



Wheat FISH karyotype

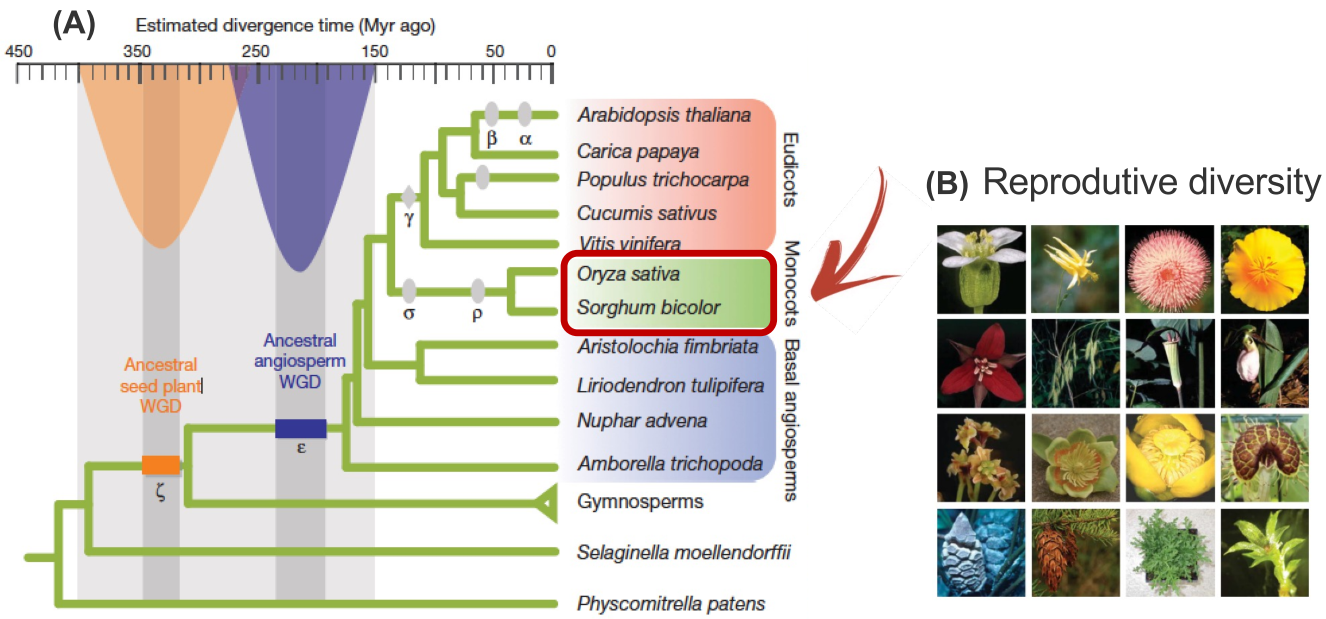
Ancestral polyploidy - **Paleopolyploidy**

- Occurred at least once during the evolutionary history of all **angiosperms**
- **Polyploidy**, followed by gene loss and diploidization, was an important evolutionary force in plants.
- **Innovations** induced associated evolutive success of angiosperms.

Ancestral polyploidy events

(A) Two ancestral duplications
- one in the ancestor of **gymnosperms**
- other in the ancestor **angiosperms**

(B) Innovations in reproductive organs



Jiao et al 2011

Cereal genome **sequencing**

SEQUENCIAÇÃO

- Principais **metodologias de sequenciação**
- Caraterização do **genoma dos trigo e espécies aparentadas**
  - **Identificação de poliploides ancestrais**

Sequencing

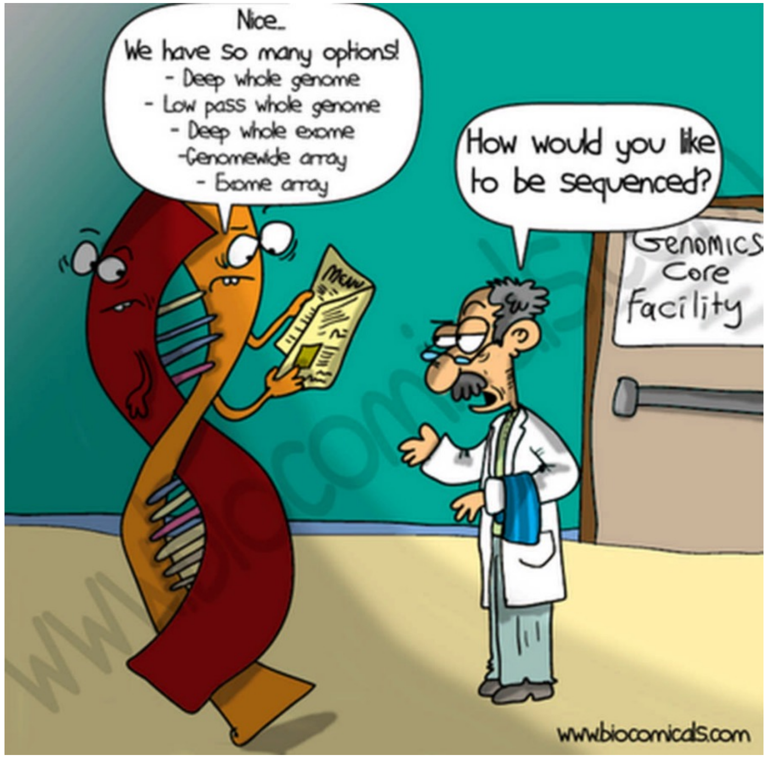
- "**Sequencing**" – to know the **order** of

  - **nucleotides** in the **DNA** molecules
  (- aminoacids in the proteins)

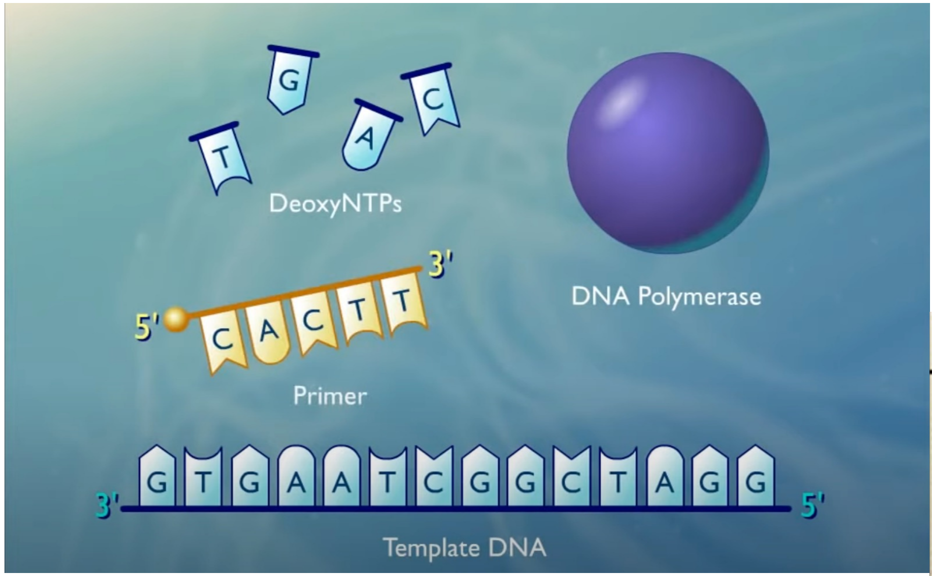
- The nucleotides order determines the amino acids order and therefore the protein structure and function

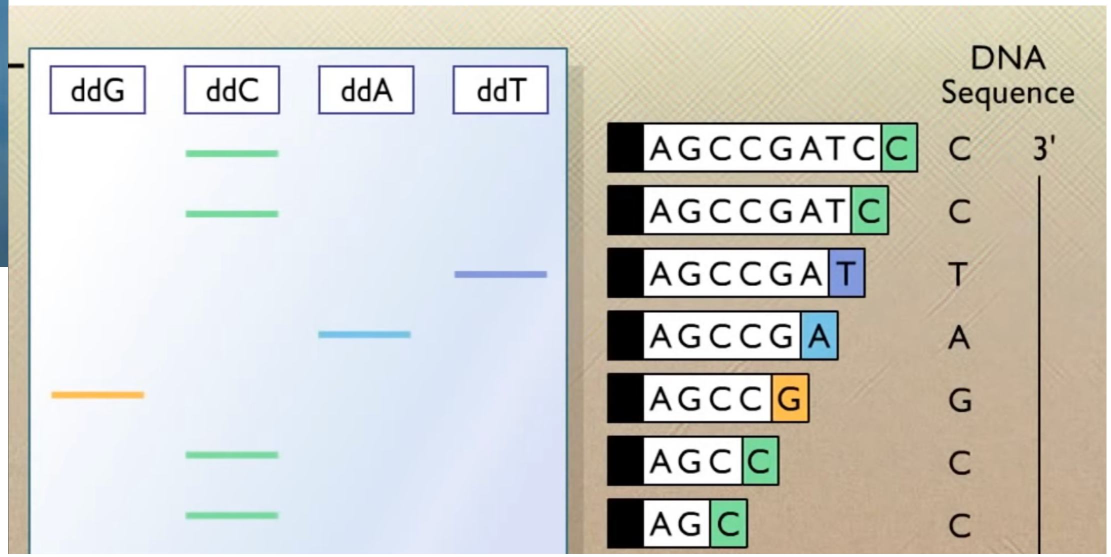
- Changes in the DNA can be correlated with changes in proteins codded in the DNA

→ Allowed the **identification on paleopolyploid species**



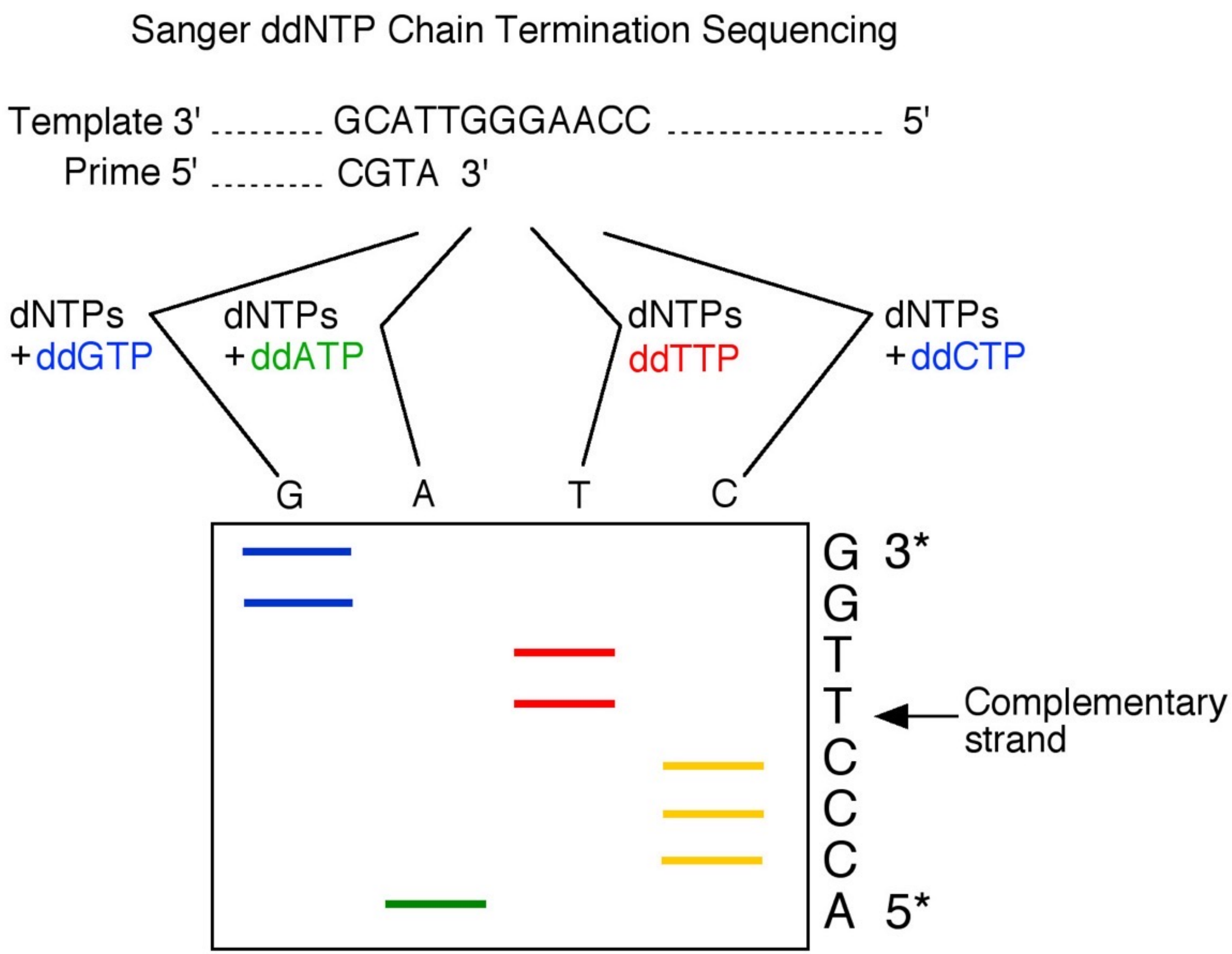
Sequenciação – Método Sanger



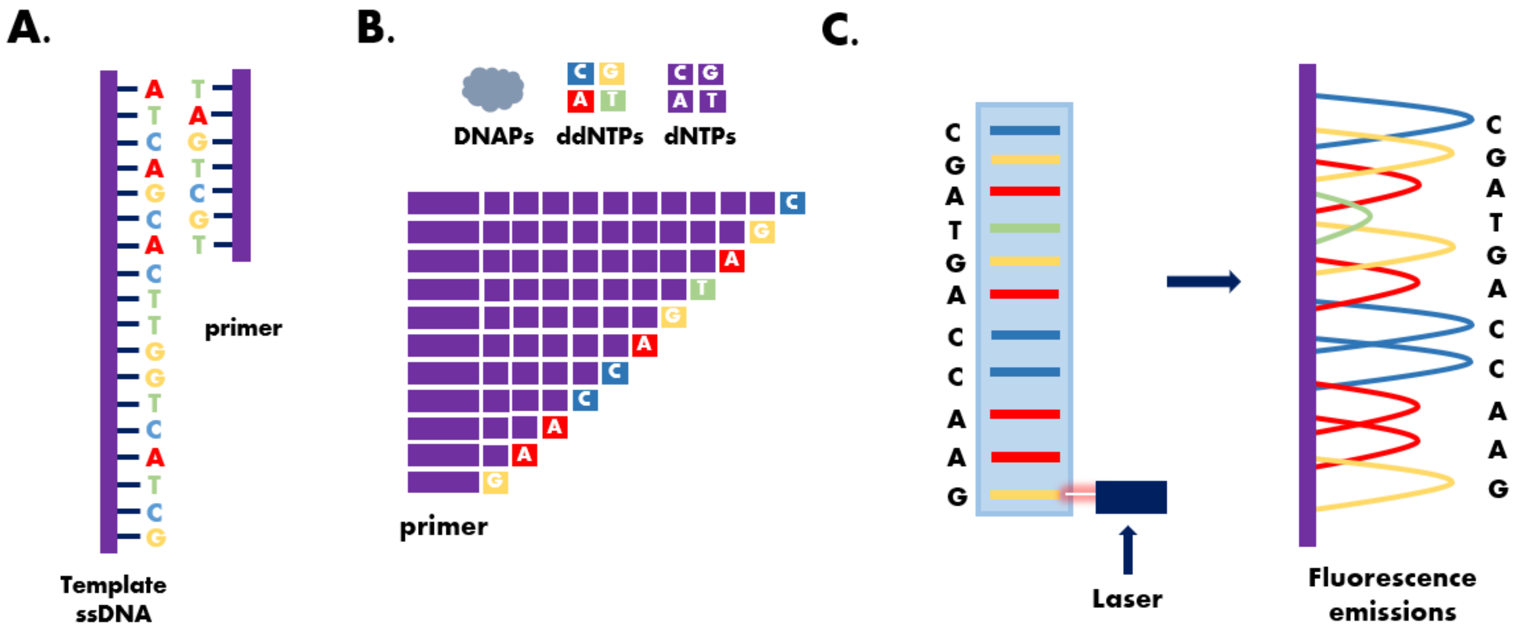


https://www.youtube.com/watch?v=AI4CnG5Jp4s&ab_channel=LaUrsa 2'22''

The Sanger method



Automated Sanger Sequencing Technology



A. The target DNA is fragmented, amplified, denatured, and bound to a primer.

B. The elongation process occurs in a single reaction mixture where the addition of fluorescently labelled dideoxynucleotides (ddNTP) results in termination.

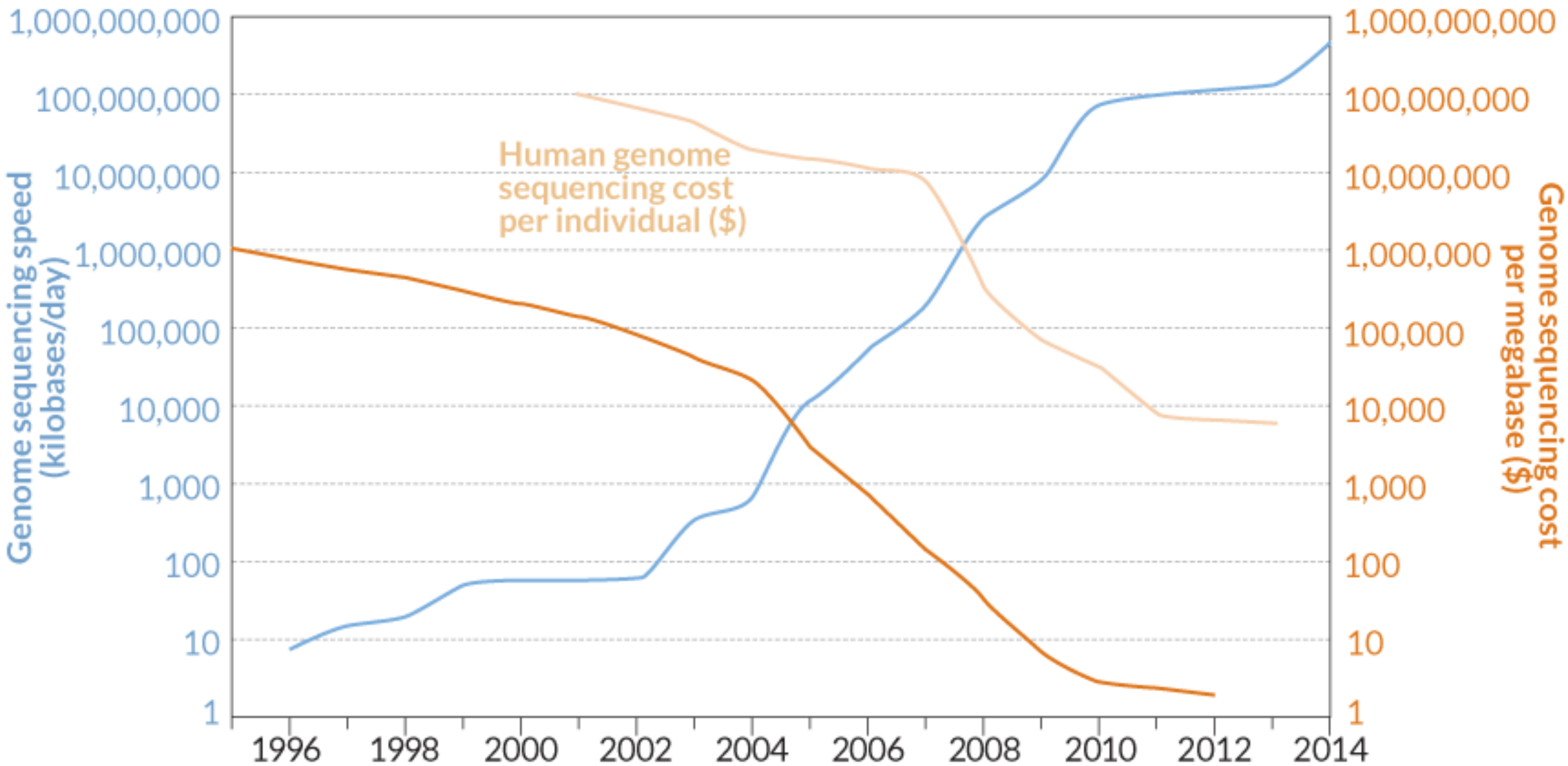
C. Capillary gel electrophoresis to separate the terminated fragments by size and laser excitation for detection.

Sanger Sequencing

Sequencing methods

Sanger	Next-generation sequencing	Third generation technologies
One DNA molecule sequenced at a time Gel Sanger Capillary Sanger	DNA is broken into **short** pieces, **amplified**, and then sequenced. Illumina GA	**Directly sequencing of** single DNA molecules. DNA do not break down or amplify. PacBio SMRT Nanopore

Genome sequencing evolution



Improvements in genome sequencing technology over the past two decades has

→ Higher **speed** and lower **cost**

Poaceae species with **sequenced genome**

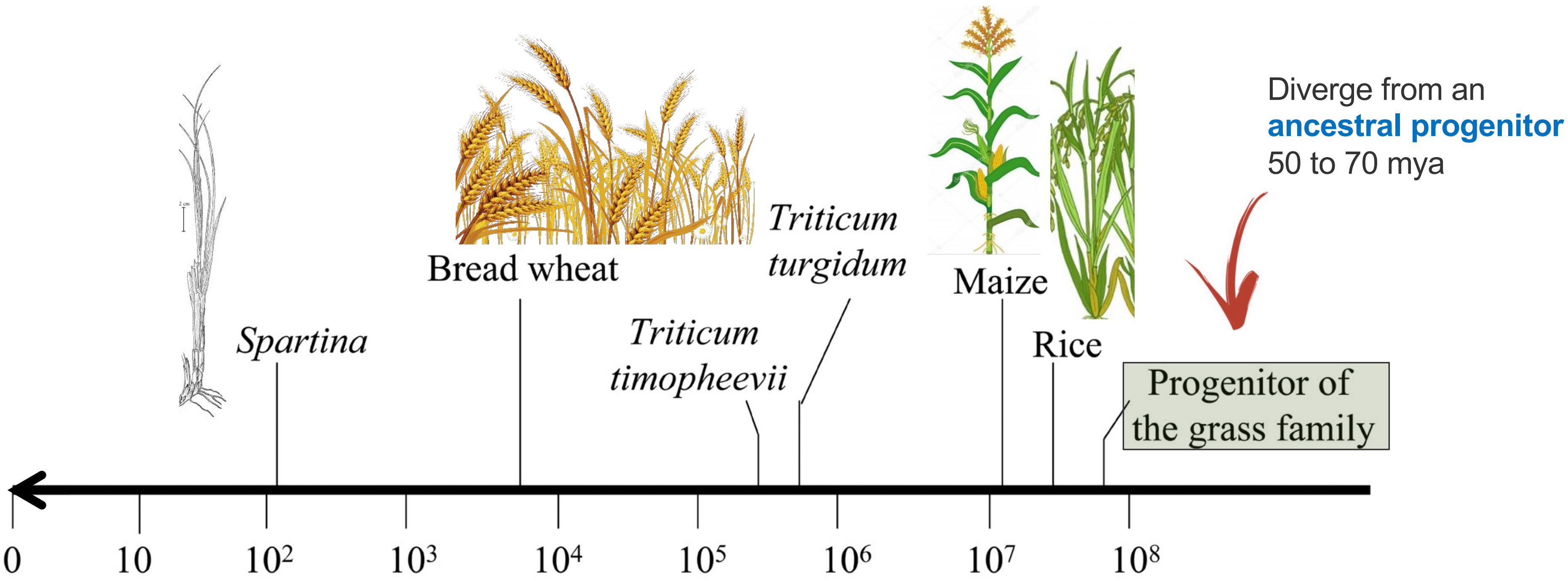
Species	Genome size	Year
Oryza sativa (long grain rice) ssp indica	430 Mbp	2002
Oryza sativa (Short grain rice) ssp japonica	430 Mbp	2002
Sorghum bicolor genotype BTx623	730 Mbp	2009
Zea mays (maize) ssp mays B73	2.3 Gbp	2009
Brachypodium distachyon (purple false brome)	355 Mbp	2010
Hordeum vulgare (barley)	5.3 Gbp	2012
Triticum urartu	4.94 Gbp	2013
Aegilops tauschii (Tausch's goatgrass)	4.36 Gbp	2017
Triticum aestivum (bread wheat)	14.5 Gbp	2018

Gramineae polyploids

Poaceae (Gramineae) is one of the largest monocots families (10,000 species, 600 to 700 genera)

→ diverged from an ancestral progenitor 50 to 70 million years ago

- **Polyploidy in grasses** is an ongoing process



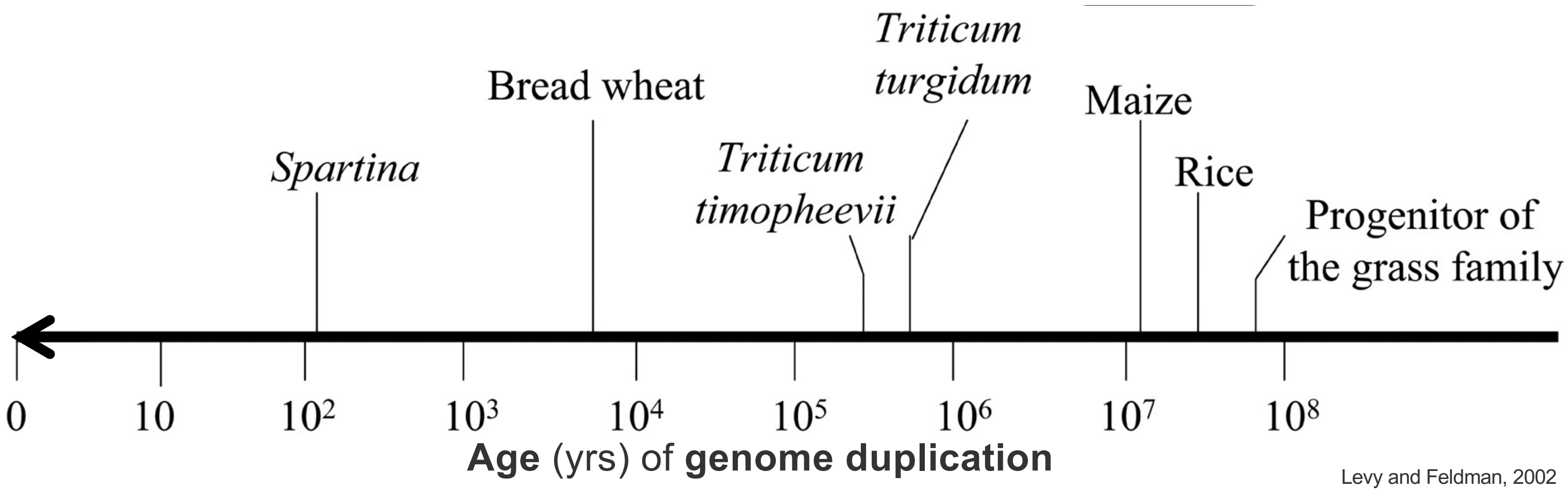
The estimated number of years since **polyploidy** occurred

Levy and Feldman, 2002

Gramineae polyploids

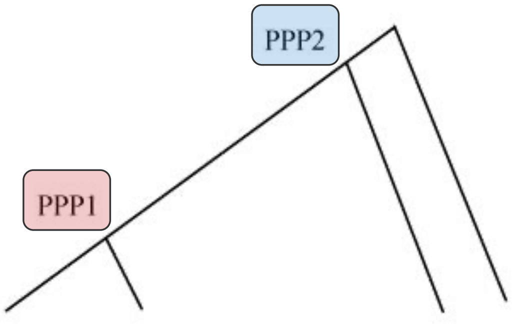
	Polyploids
Spartina	100
6x Wheat	~10 000
4x Wheat	500 000
	Paleopolyploids
Maize	11 000 000
Rice	50 000 000

Cereal species selection
- **domestication**
- **improvement**



Levy and Feldman, 2002

Rice (*Oryza sativa*) – ancient polyploid



Rice genome analysis

PPP1 - ancient genome duplication that precedes divergence of **cereals**

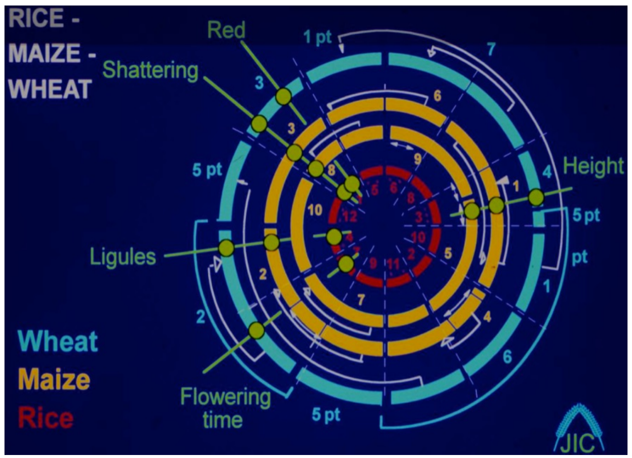
PPP2 - another <u>older</u> large-scale duplication event that precedes **monocotdicot** divergence

The **first** genome of a crop plant that was completely **sequenced** in 2002

- Sequencing of two major **subspecies -** indica & japonica

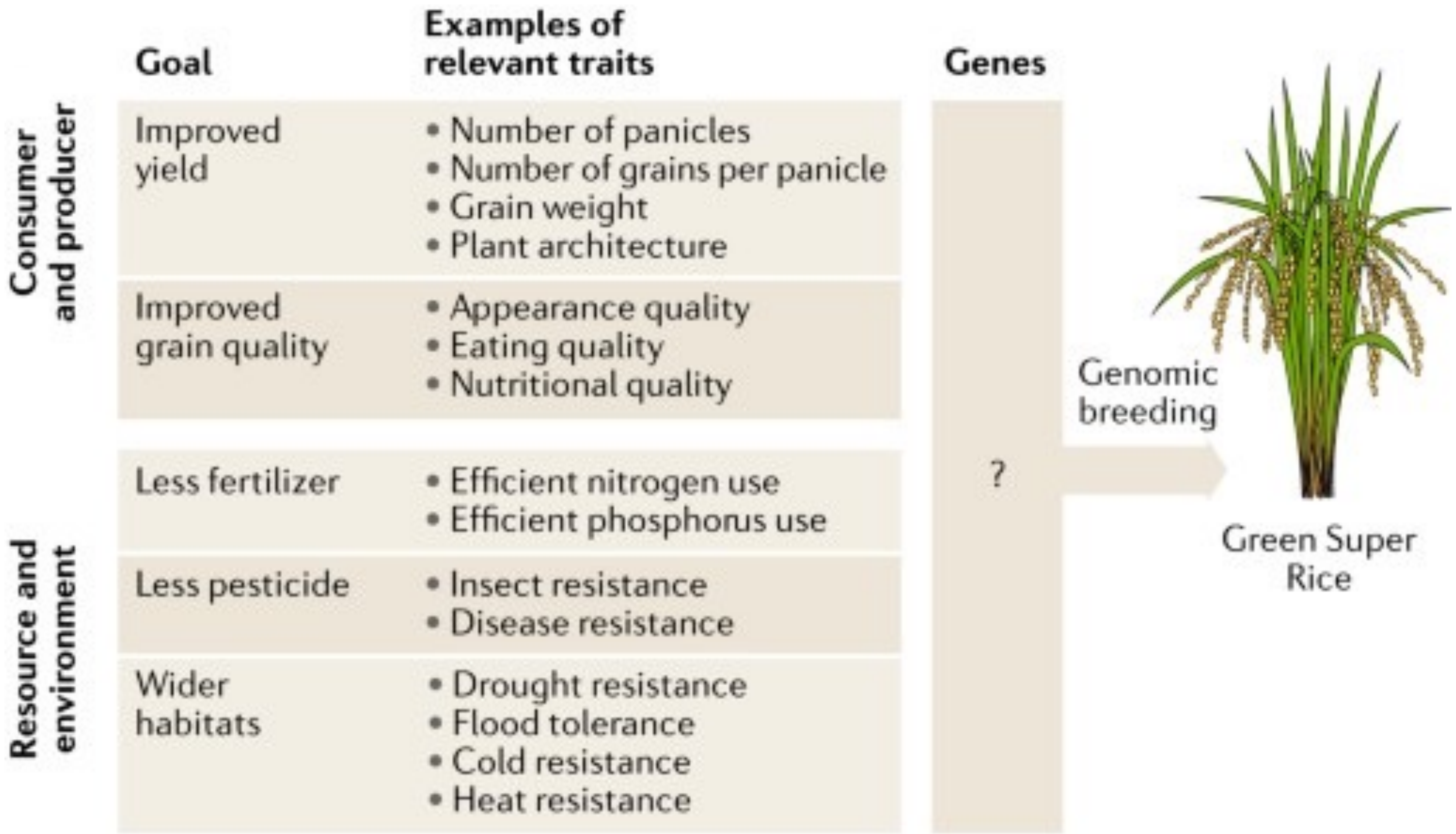
- Model cereal crop **- small genome** size (2n = 24)

- High degree of **colinearity with other cereals** genome
  Ex: wheat, barley and maize



Zhang et a 2005

Rice – from ancient polyploid to breeding

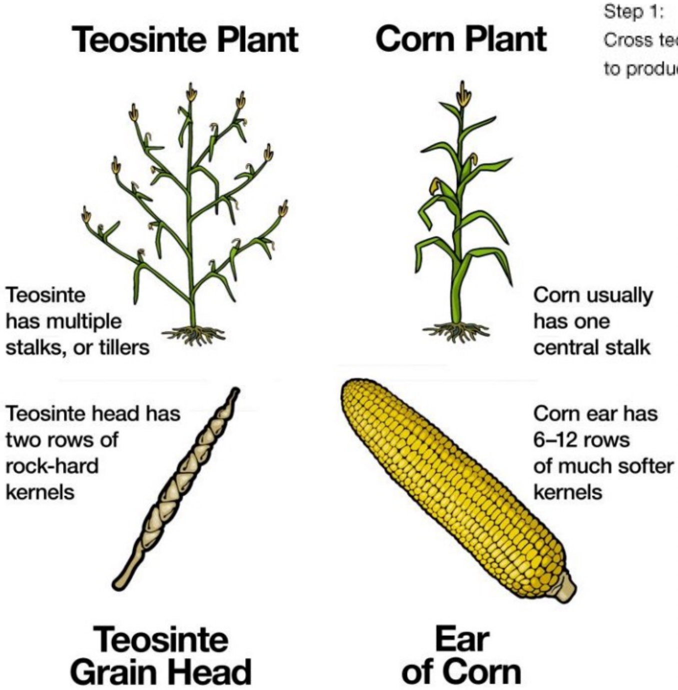


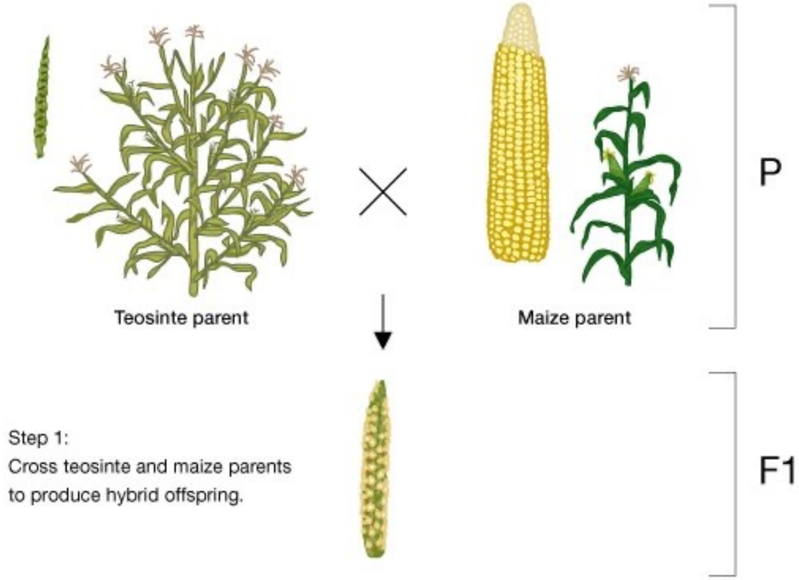
→ **Genetic variation** among domesticated rice species and their **wild relatives** has been investigated to identifying traits that can be exploited for breed

Wing et al 2018

Evolution of **maize**

Maize cobs uncovered by **archaeologists** show the evolution of modern maize over thousands of years of **selective breeding**.

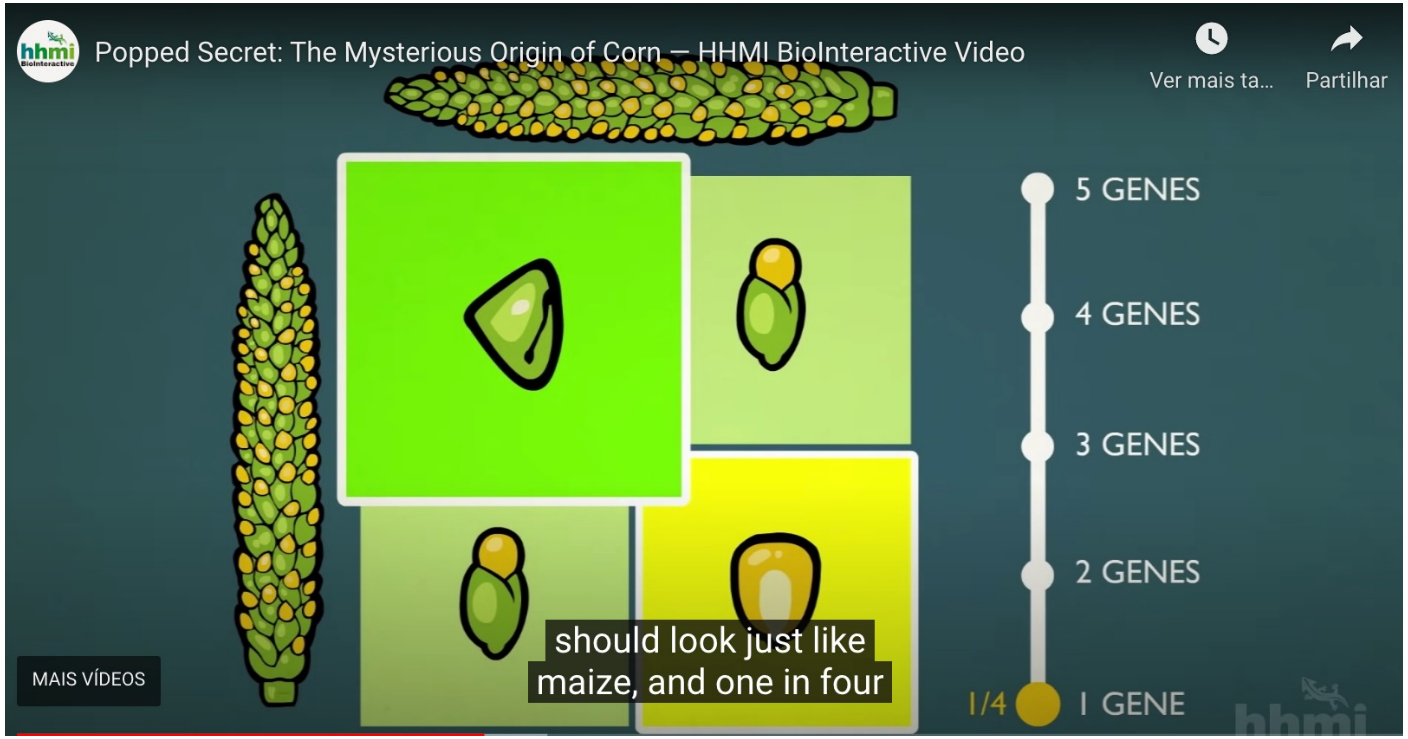




George Beadle 1930s - **teosinte-maize hybrids** → chromosomes are highly compatible.

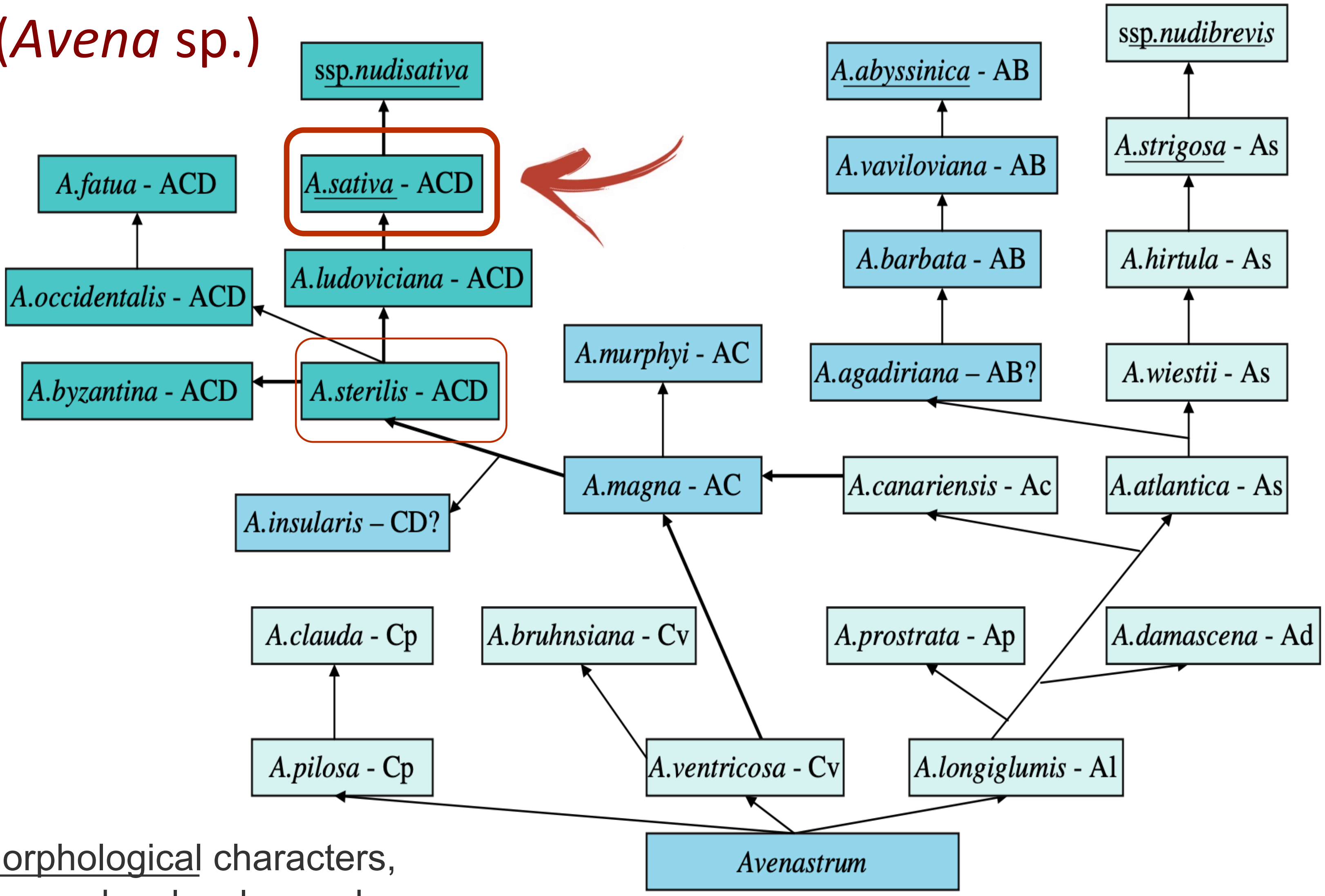
Calculated that only about **5 genes** were responsible for the notable differences between teosinte and a primitive strain of maize.

Popped Secret: The Mysterious Origin of **Corn**



https://www.youtube.com/watch?v=mBuYUb_mFXA&ab_channel=biointeractive 17’52”

Oat evolution (*Avena* sp.)



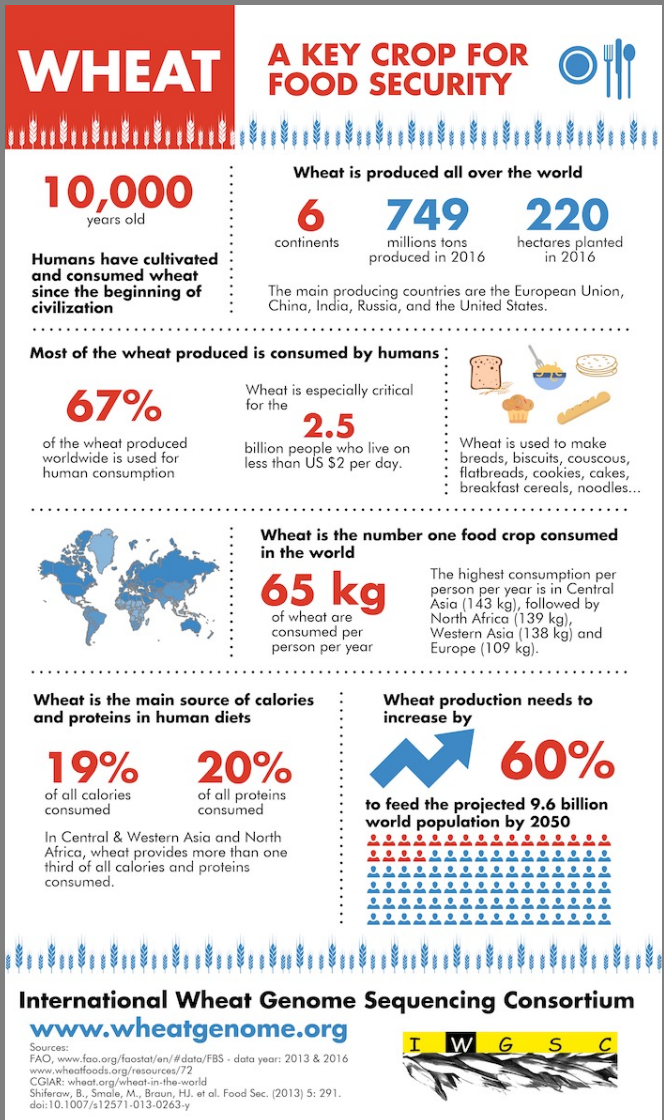
Study of major morphological characters, karyotype structure and molecular markers

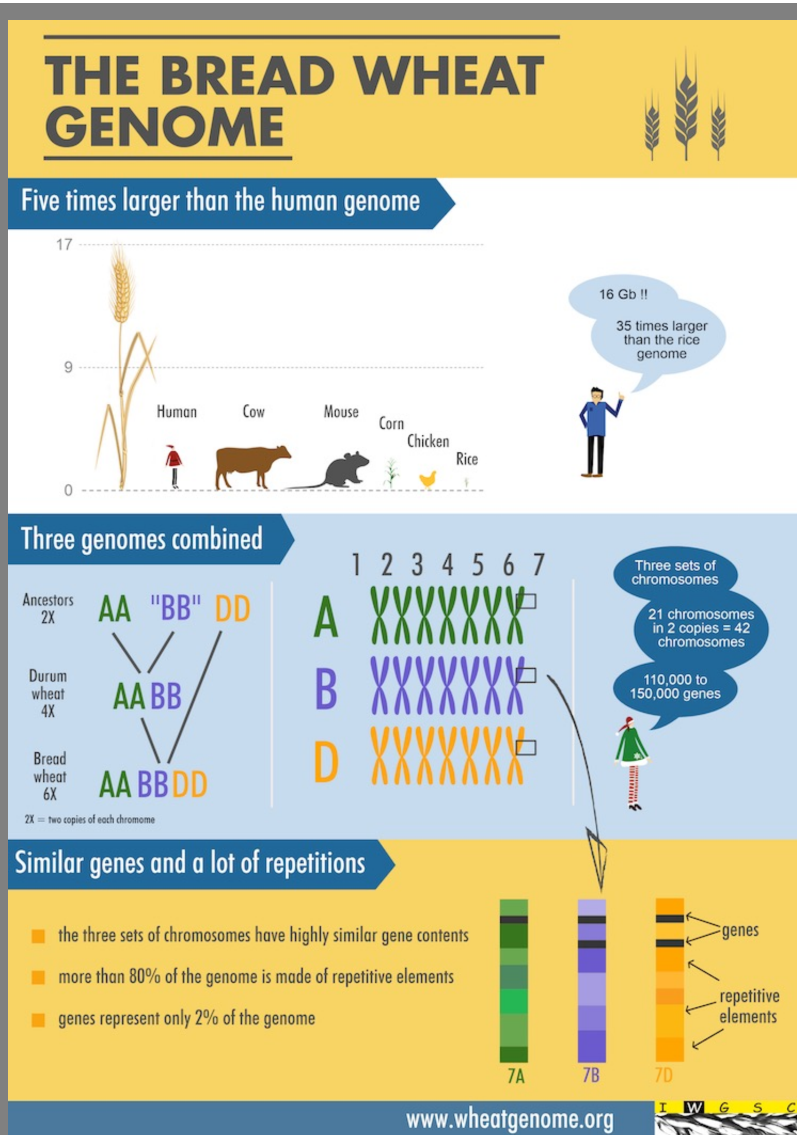
2008 Loskutov

International **Wheat** Genome Sequencing Consortium **IWGSC**

Production must increase 60% until 2050

Human main source of calories and protein

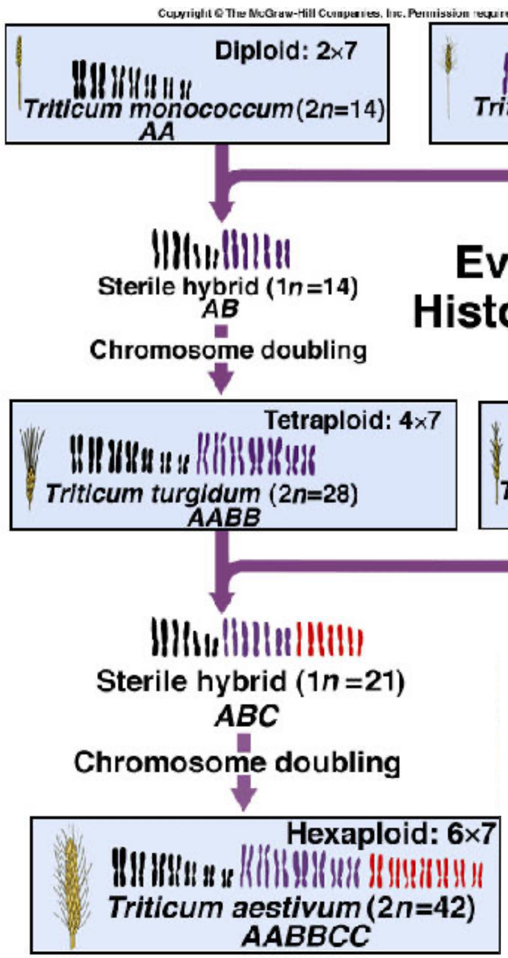




Wheat genome size **35x** rice genome

Complex hexaploid genome

Wheat (*T. turgidum, T. aestivum*) evolutive history



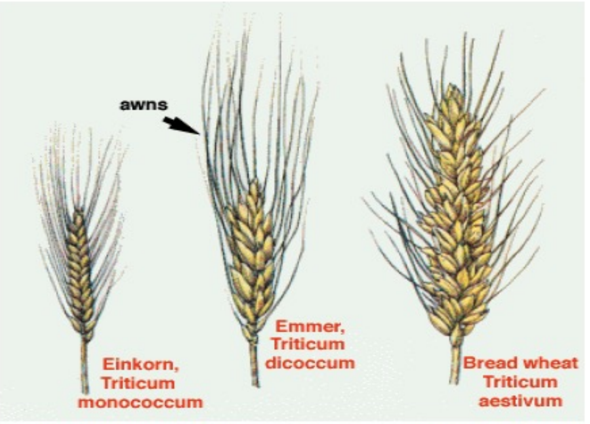
Wheat <u>polyploidization</u> events:

500 000 years ago

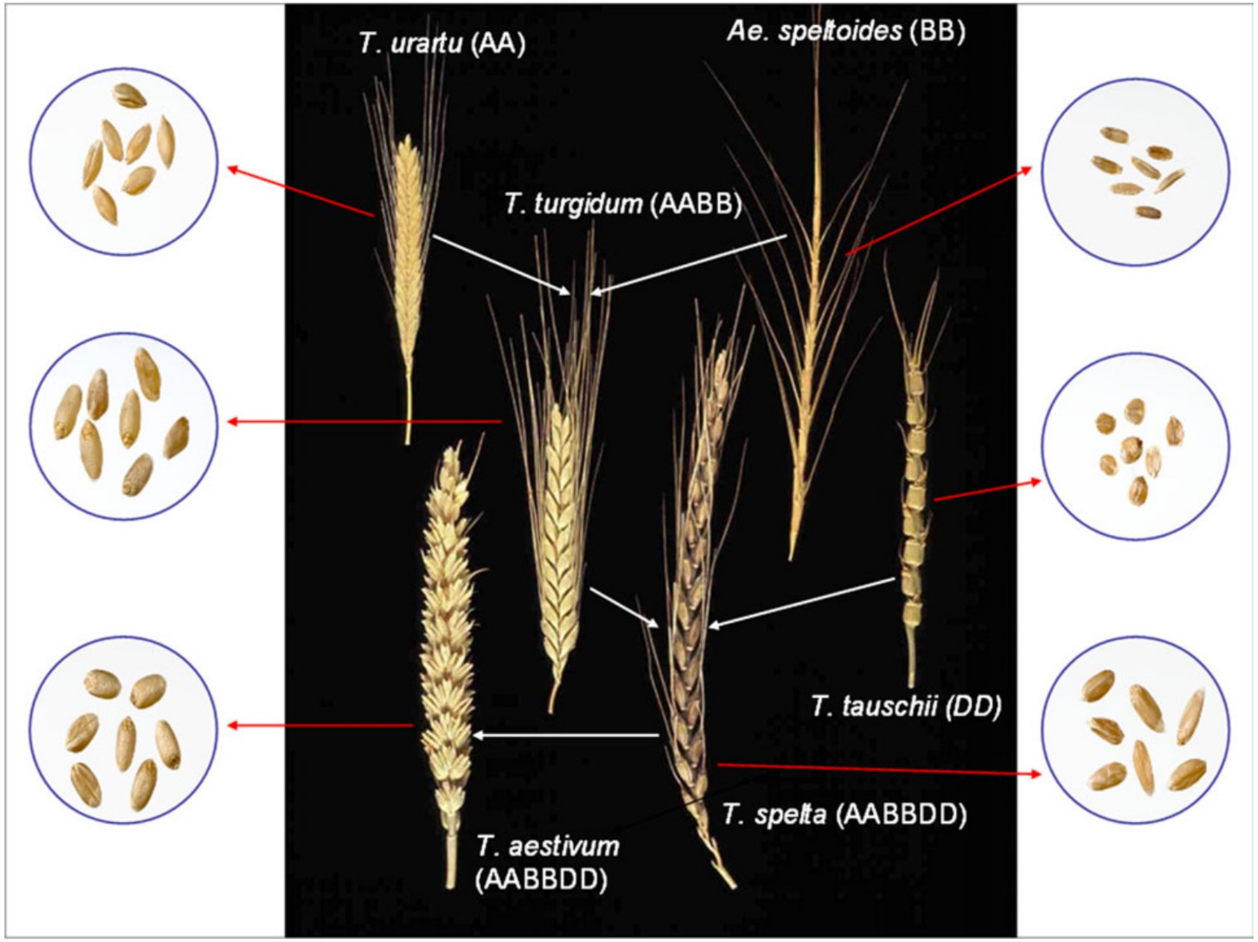
- **durum wheat** (tetraploid, 2n = 28)

8000–10 000 years ago

- **bread wheat** (hexaploid, 2n = 42)



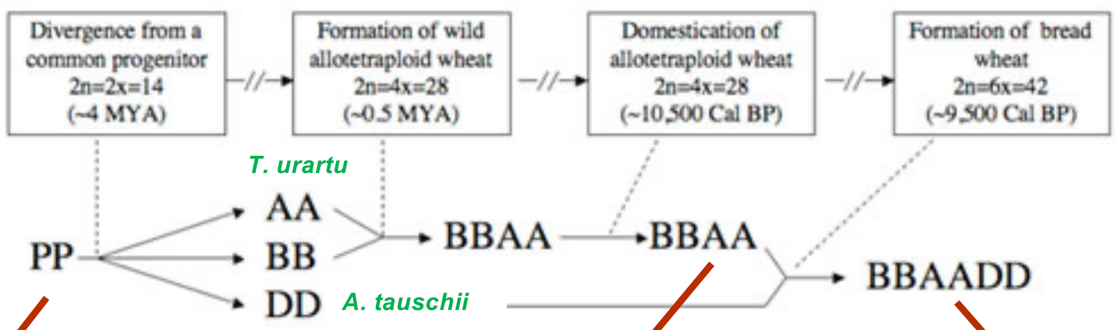
Wheat and relative species - spikes and grains



Shewry 2009

Wheat evolution through **polyploidization**

Evolutionary history of wheat



Common Ancestor
(2n=2x=14, diploid)

Durum wheat

(2n=4x=28, tetraploid)

Bread wheat

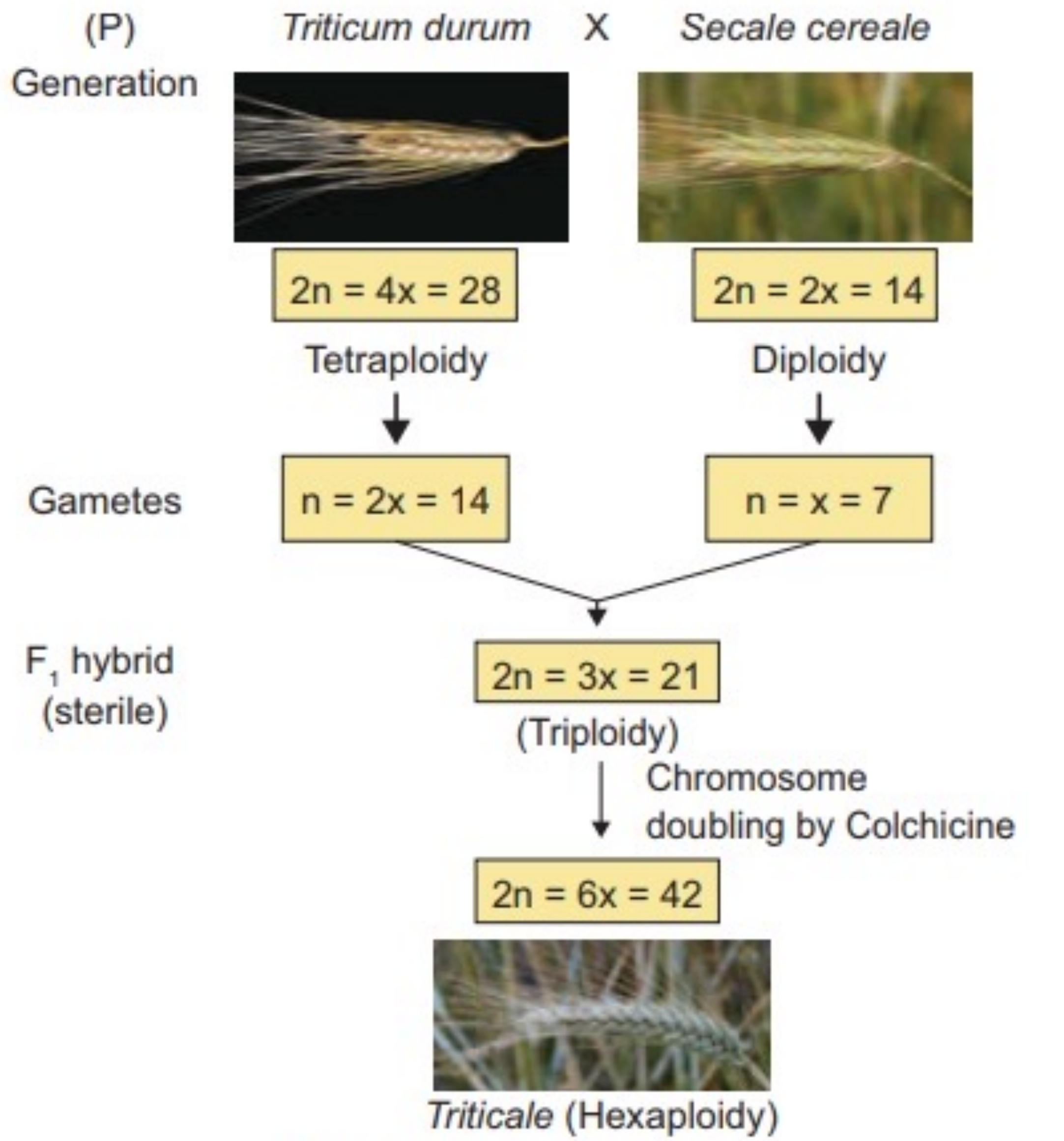
(2n=6x=42, hexaploid)

Levy 2007

Evolutionary History of Wheat

https://colostate.pressbooks.pub/cropwildrelatives/chapter/wheat-breeding-with-crop-wild-relatives/

Synthetic allopolyploid: **triticale**



Seeds of wheat (left), triticale (centre) and rye (right)
(Source: A. Stephen Wilson 1875)

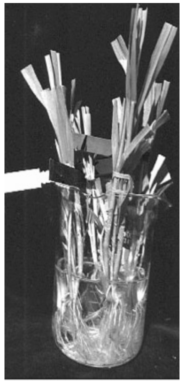
Artificial methods to induce polyploidy: **chromosome duplication**

- **Colchicine** - inhibits microtubule polymerization by binding to the main constituents of microtubules – tubulin

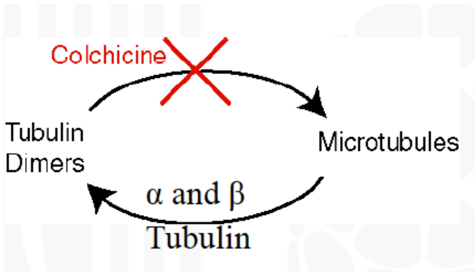
→ **blocking of mitotic spindle formation**

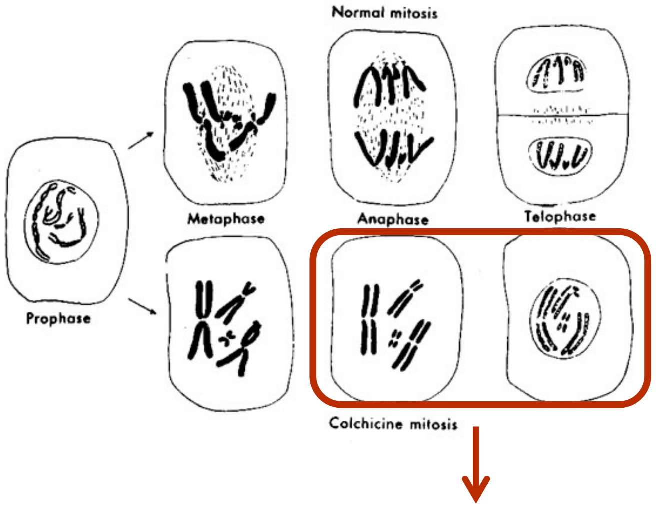
Cells cannot split into two daugther cells

Colchicum autumnale



Colchicine treatment

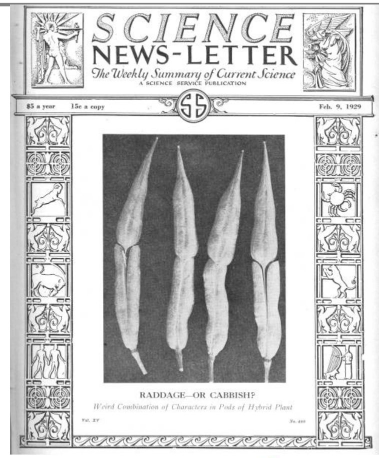




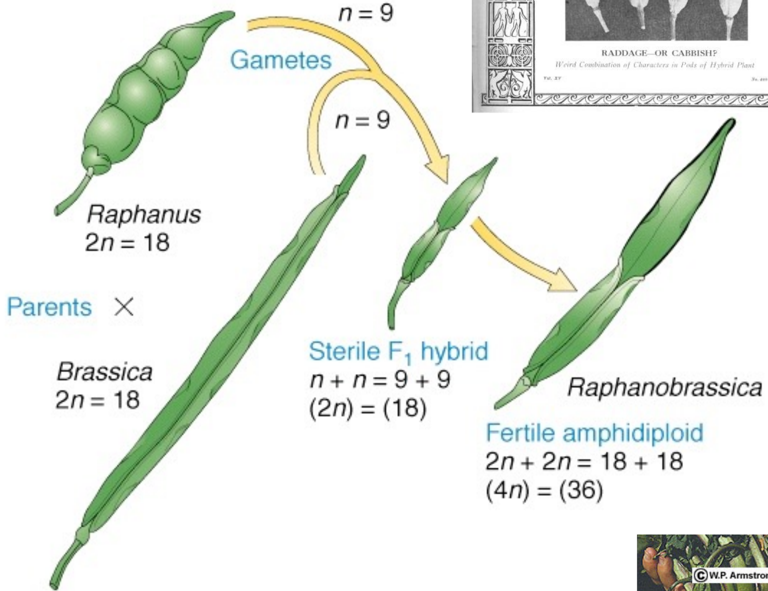
Chromosome duplication

Polyploids induced by colchicine

1928 – Karpechenko G. D. produced the **first artifitial polyploid** plant



Raphanobrassica



R sativus **X** *Brassica* → **sterile** hybrid

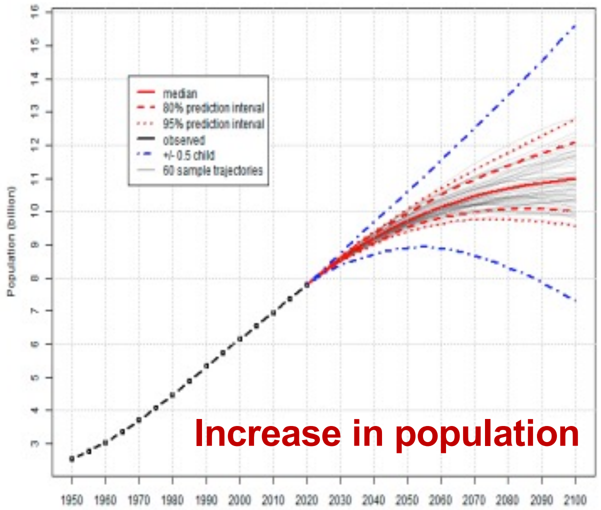
Colchicine treatment → **fertile** polyploid

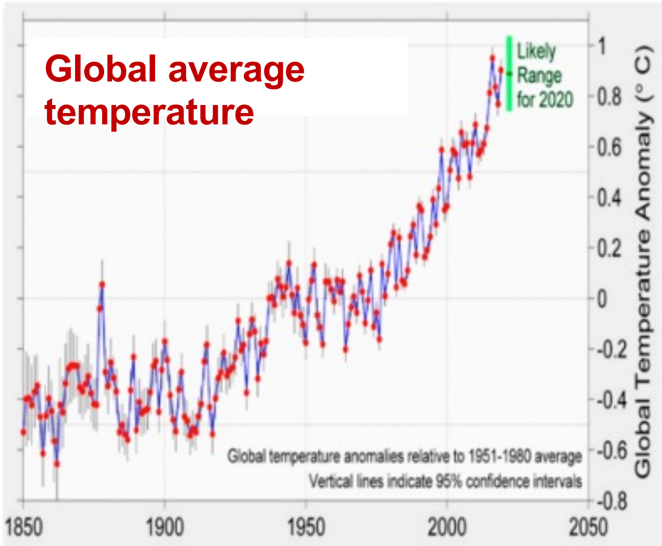
But…

Brassica root and *Raphanus* leaf

Raphanus sativus X Brassica

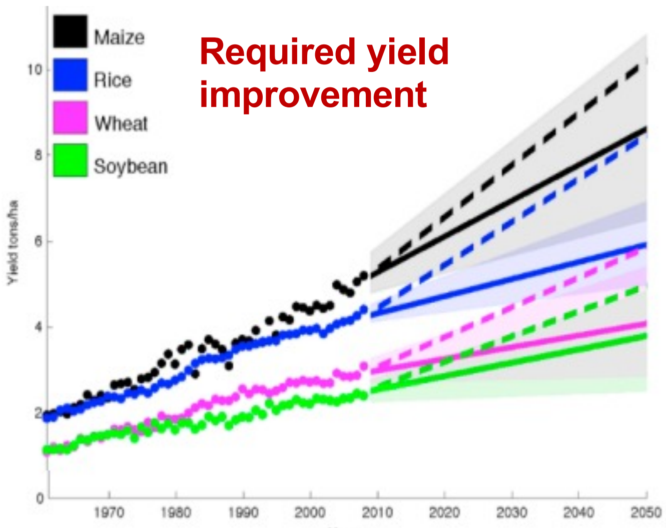
Future increase in food demand





The most sustainable path to achieve food security is by **increasing crop yields** <u>instead of use more land</u>.

Improved plants are being developed through the application of **advances in genetic technologies**.



Future of Cereal Genomics and Breeding

Genomic knowledge combined with traditional breeding methods to increase cereal crop production and resilience

→ Commercial varieties

→ **Wild relatives**

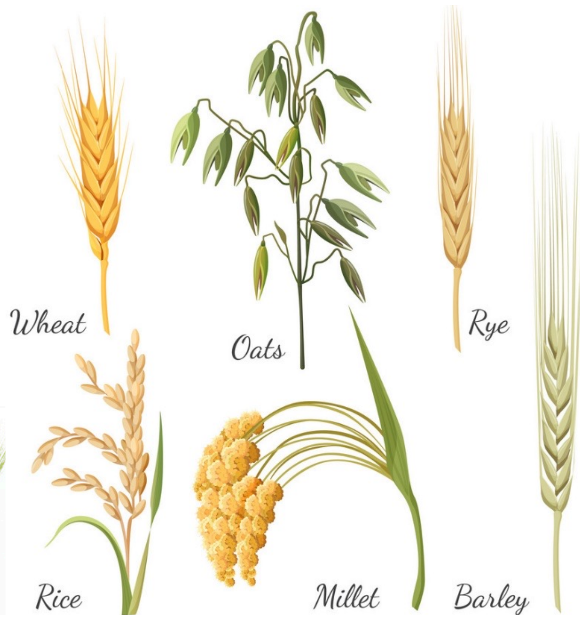
→ **Old traditional varieties**

Landraces assume crucial importance as pools of **agrobiodiversity** of

- useful traits for wheat breeding
- pre-adapted to extreme environmental conditions

Particularly considering the **genetically eroded commercial varieties**

← decades of homogenization through breeding



Colecção de variedades tradicionais de trigo mole e trigo duro

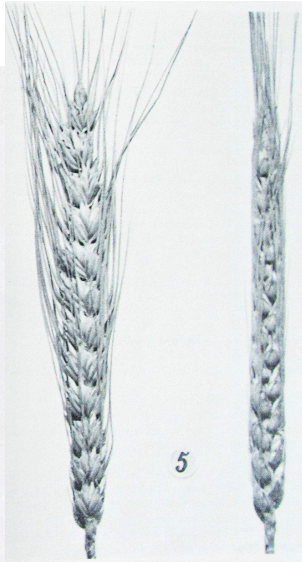

Boletim de Agricultura

ANO I—MARÇO e JUNHO, 1933

NÚMEROS I e 2—I SÉRIE

Editado pelo SERVIÇO DE PUBLICIDADE E BIBLIOTECA da DIRECÇÃO GERAL DA ACÇÃO SOCIAL AGRÁRIA
MINISTÉRIO DO COMÉRCIO, INDÚSTRIA E AGRICULTURA

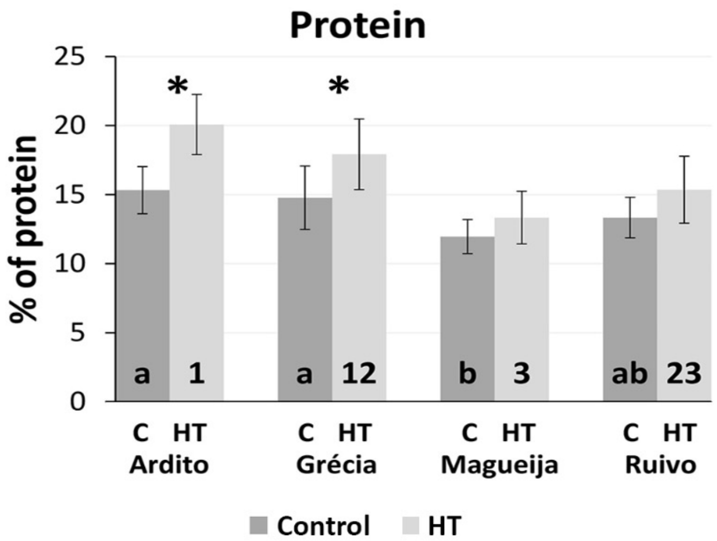
TRIGOS PORTUGUESES
OU DE HÁ MUITO CULTIVADOS NO PAÍS
(SUBSÍDIOS PARA O SEU ESTUDO BOTANICO)
por
JOÃO DE CARVALHO E VASCONCELOS
Engenheiro agrónomo em serviço na Divisão de Ensaio de Sementes
e Melhoramento das Plantas da Estação Agrária Central

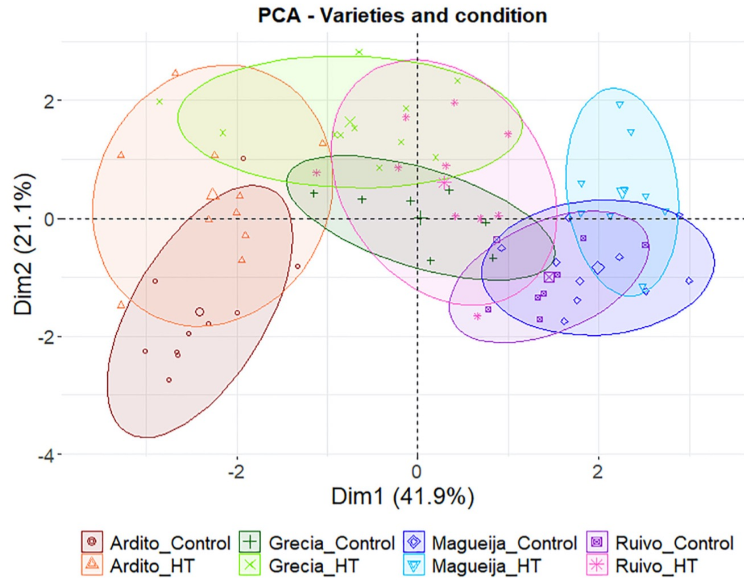


Vasconcelos, J. C. (1933). **Trigos portuguêses ou de há muito cultivados no país**. Subsídios para o seu estudo botânico. *Bol. Agric.* 1, 2, 1–150.

Assessment of Portuguese bread **wheat landrace** diversity to cope with **global warming**

Evaluation of heatwave like treatment effects on Portuguese landrace yield and grain composition





Heatwave treatment → general **increase** in grain **protein** content

But… **Landraces** showed **variability** in:

- **Yield** traits (grain number and weight)
- Grain major components

→ **protein** content and **polysaccharide** composition

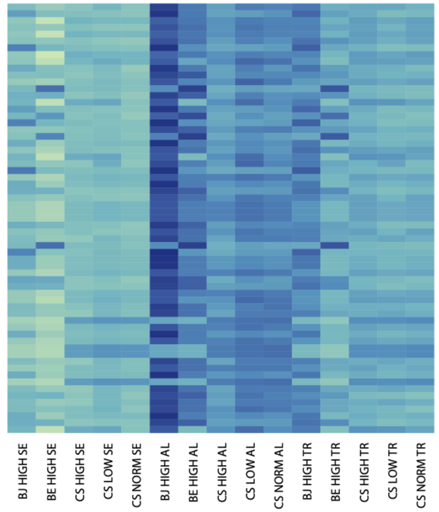
Integrated assessment
→ distinct responses to cope with heat

Tomás et al 2020

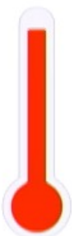
Heat effect on **wheat** grain
- level of peptides involved in **celiac disease**

Evaluation of expression level of 63 genes coding peptides with known immunoreactivity

high temperature → increased expression levels



Climate change does impact wheat allergen expression

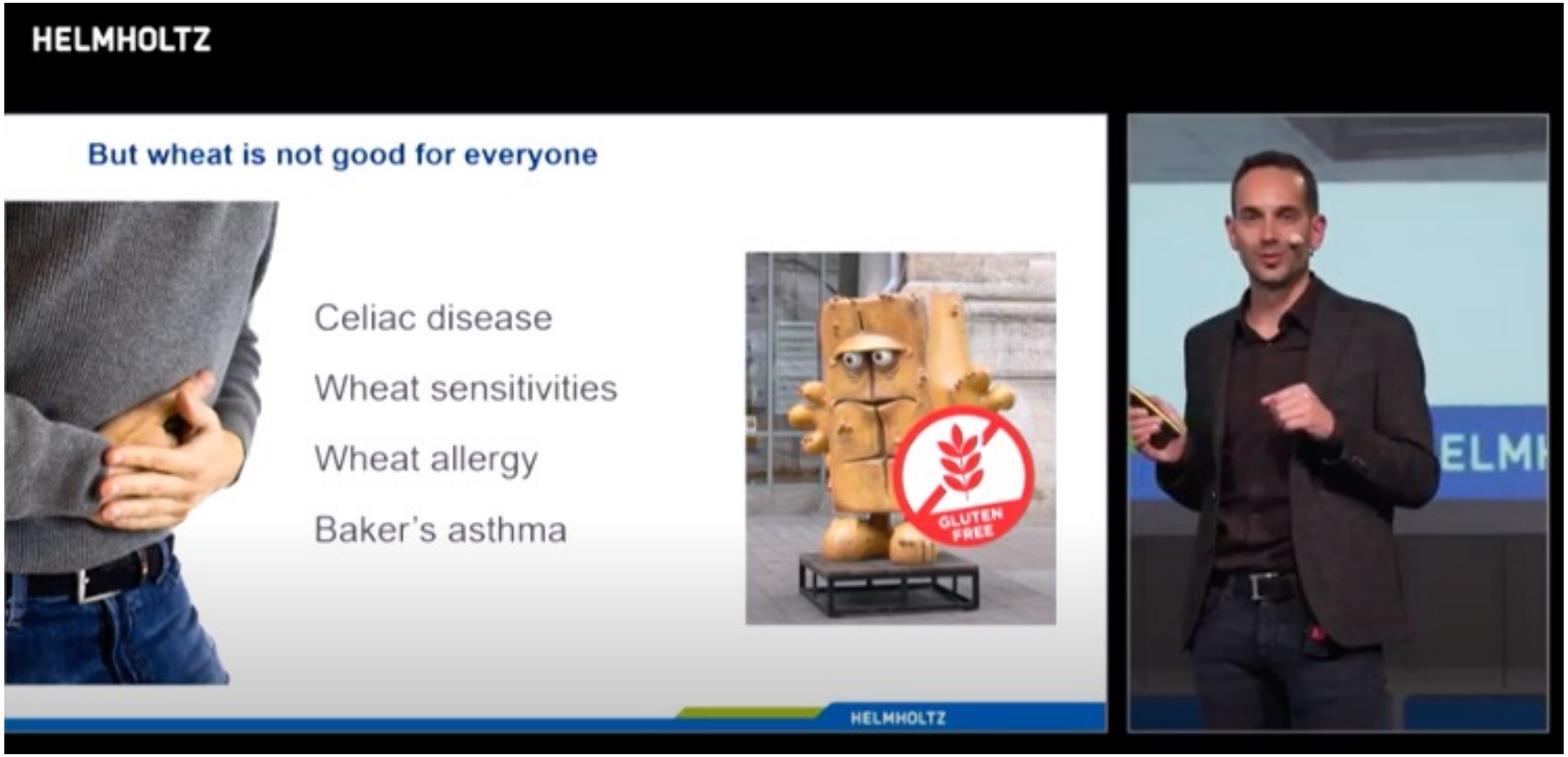


High temperature

Increasing effect on the celiac disease associated gene expressions – gluten proteins

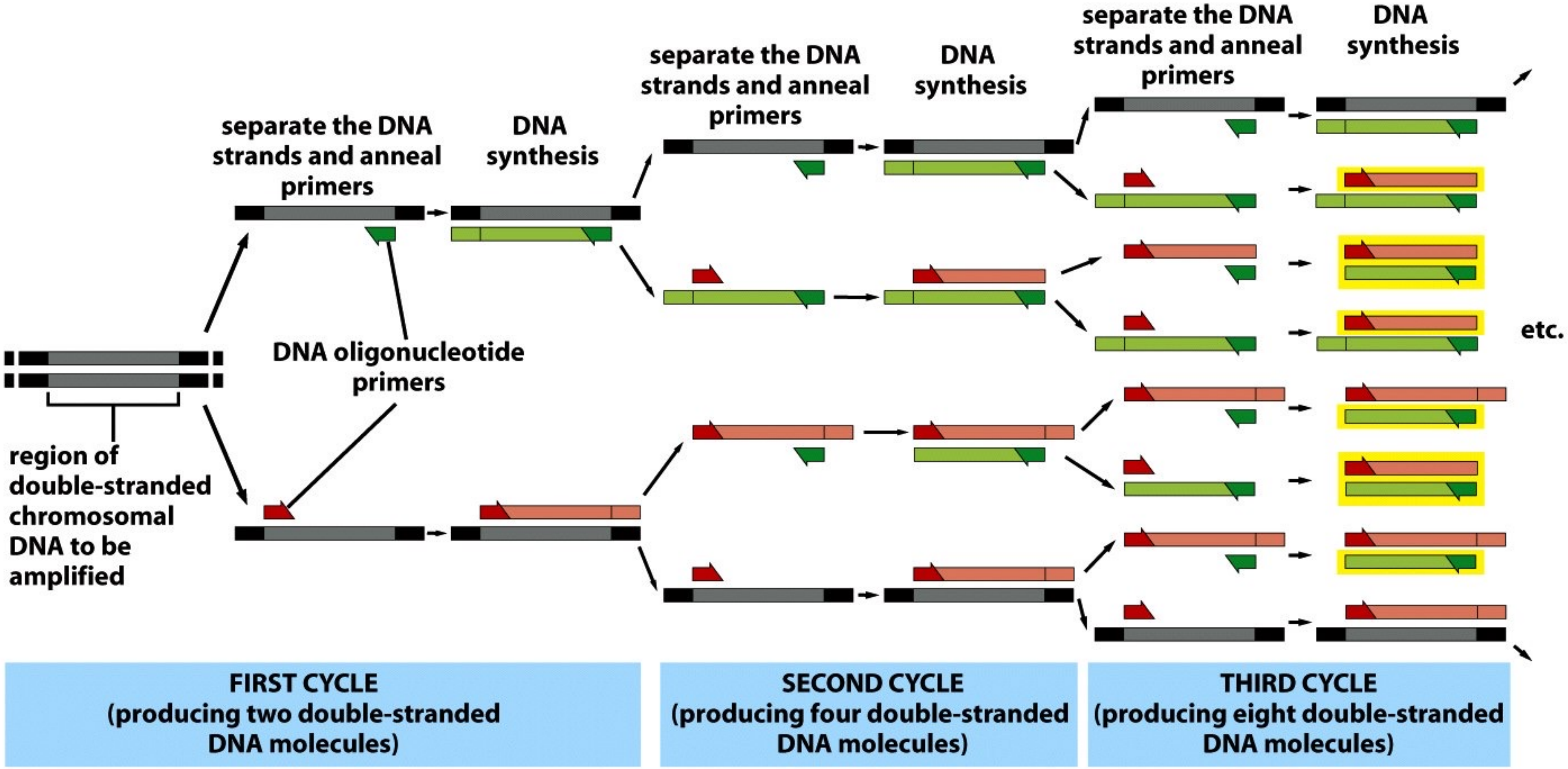
2018 Juhász et al

The heat is on: of cereals and genome



https://www.youtube.com/watch?v=6I1dU2-tdVU

PCR (Polimerase Chain Reaction)



Amplificação de DNA - em cada ciclo a sequência localizada entre os dois *primers* originais é copiada (DNA sintetizada pela DNA Taq polimerase).

Figure 8-45b *Molecular Biology of the Cell* (© Garland Science 2008)

Genome size

Genome - entire DNA complement of an organism

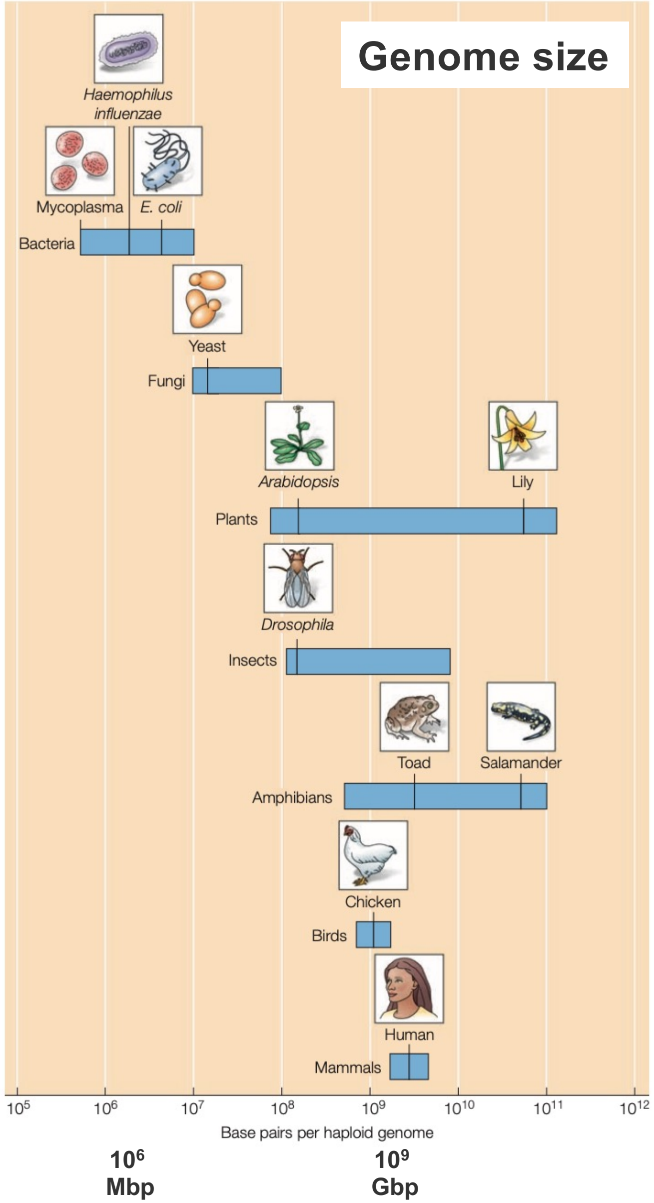
Eukaryote genomes
= DNA regions encoding proteins – **genes** (~ 1.5%)
+ apparently **nonfunctional repetitive** DNA

Amphibia - vertebrates with the greatest genome
Plant species - considerably more DNA than humans
ex: tulips 10x

DNA content varies considerably also between **closely related species**

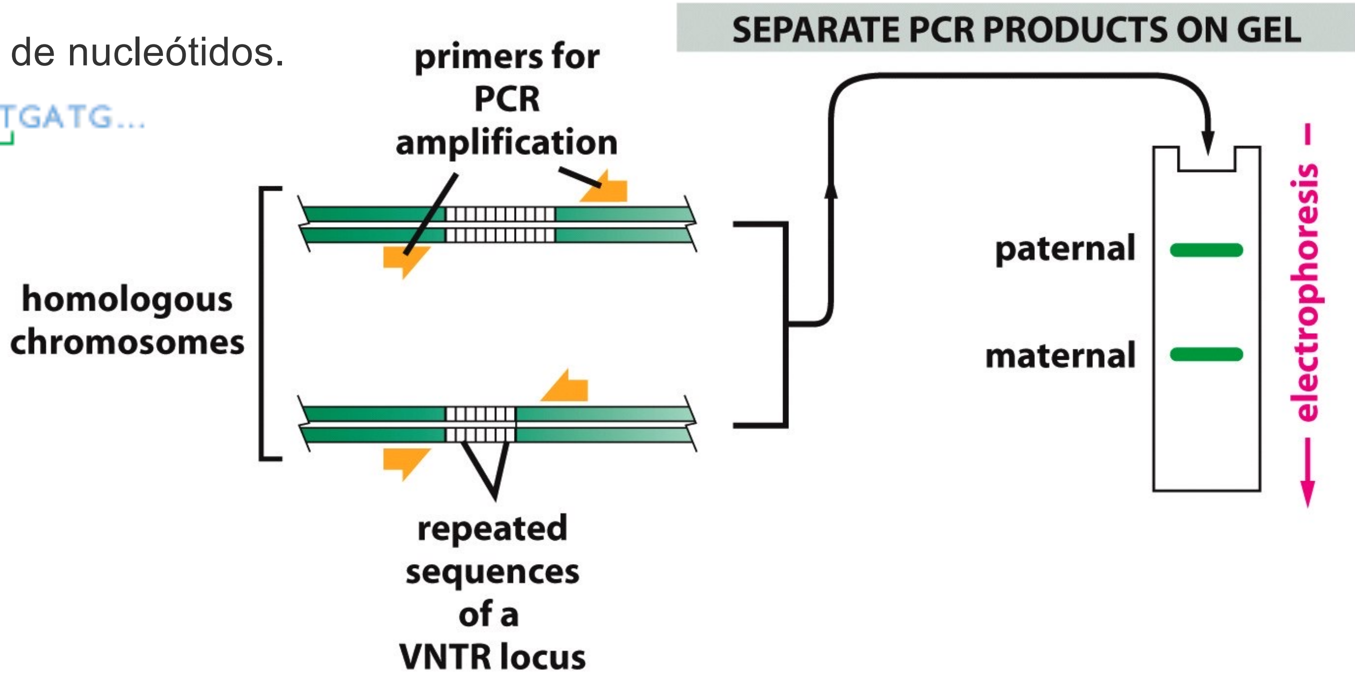
Ex: insects or amphibians
100x in species within each of these phylogenetic classes, although similarly complex

The Cell: A Molecular Approach. 8th Edition



Caracterização de um genoma
- detecção de mutações/polimorfismos do DNA por PCR

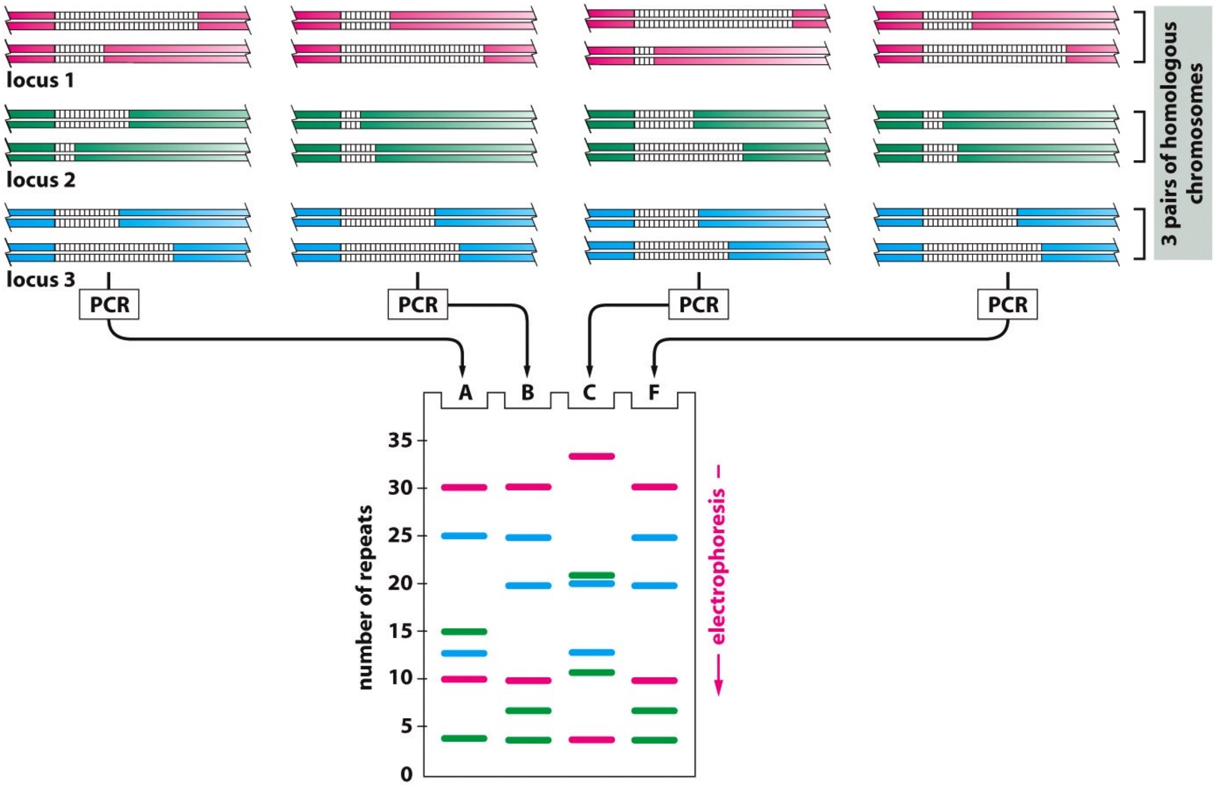
Microsatélies – sequências repetidas de nucleótidos.



- **Polimorfismos** - pequenas variações (mutações) no nº de nucleótidos
- <u>Análise dos polimorfismos</u>:
  - amplificação do DNA com *primers* para as regiões adjacentes
  - análise da dimensão dos fragmentos de DNA amplificados por electroforese

Figure 8-47a *Molecular Biology of the Cell* (© Garland Science 2008)

Caracterização da variabilidadeatravés da análise de microsatélites



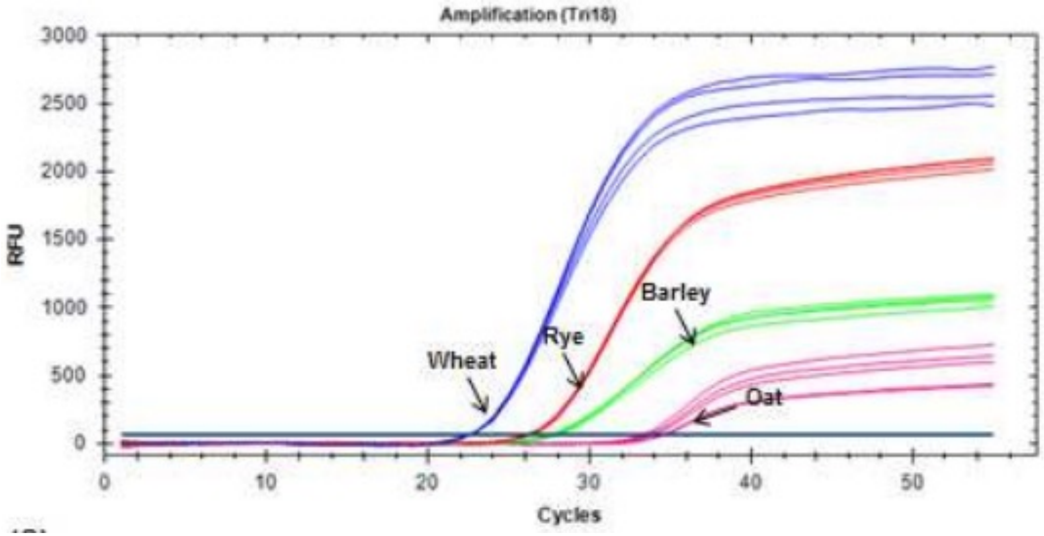
O nº de repetições nos diversos microsatélites é muito variável (4-40)
(entre espécies diferentes ou mesmo entre indivíduos da mesma espécie.

Figure 8-47b *Molecular Biology of the Cell* (© Garland Science 2008)

Food Control
Volume 56, October 2015, Pages 57-63

Screening new gene markers for gluten detection in foods

Begoña Martín-Fernández [a, b], Joana Costa [a], M. Beatriz P.P. Oliveira [a], Beatriz López-Ruiz [b], Isabel Mafra [a]



Amplification obtained by real-time PCR of α2-gliadin in DNA from wheat and related cereals containing gluten (barley and rye) and oat.

Detection of gluten in foods

Quantitative real-time **PCR** methods targeting **α2-gliadin** coding sequences
→ successfully detection **wheat** DNA.

Limit of detection: absolute 2 pg and relative 0.005% (50 mg/kg) of wheat in soybean (corresponding to 4.5 mg/kg of gluten).

This methodology reveals also high specificity for detecting other gluten-containing cereals, such as barley and rye.

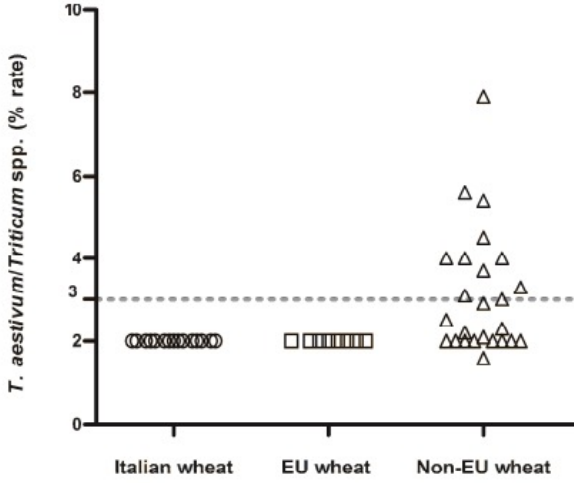
→ This PCR systems can be used as tools to confirm the presence of **gluten-containing cereals** in foods, towards the safety of celiac patients

ELSEVIER
Food Chemistry
Volume 224, 1 June 2017, Pages 86-91

Validation and application of a quantitative real-time PCR assay to detect common wheat adulteration of durum wheat for pasta production

Elisa Carloni [a], Giulia Amagliani [a], Enrica Omiccioli [b], Veronica Ceppetelli [b], Michele Del Mastro [c], Luca Rotundo [a], Giorgio Brandi [a], Mauro Magnani [a]

Italian pasta certification



→ **3%** legal Italian limit

Quantification of *T. aestivum* in Italian, European Union (EU), and Non-EU semola

Manufactured using durum wheat semolina

Italian national legislation excludes the use of bread wheat in pasta permitting a maximum content of **3%**.

→ the protection of traditional pasta requires a sensible **PCR**-related techniques

New molecular quantification method (DNA extraction from semolina)

real-time PCR targeting **gliadin** and **glutenin** genes

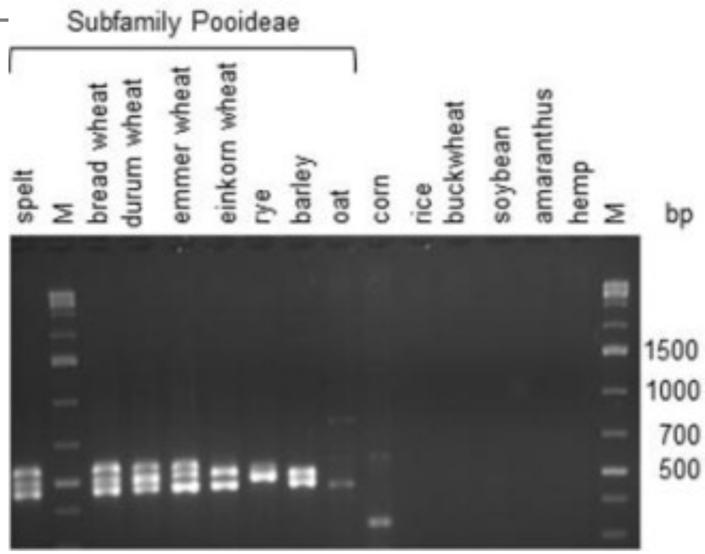
→ allow a specific and sensitive detection

ELSEVIER

Food Chemistry
Volume 271, 15 January 2019, Pages 410-418

Untargeted DNA-based methods for the authentication of wheat species and related cereals in food products

Silvia Silletti [1], Laura Morello [1], Floriana Gavazzi, Silvia Gianì, Luca Braglia, Diego Breviario



PCR amplification (TBP) on different cereal species

New food commodities

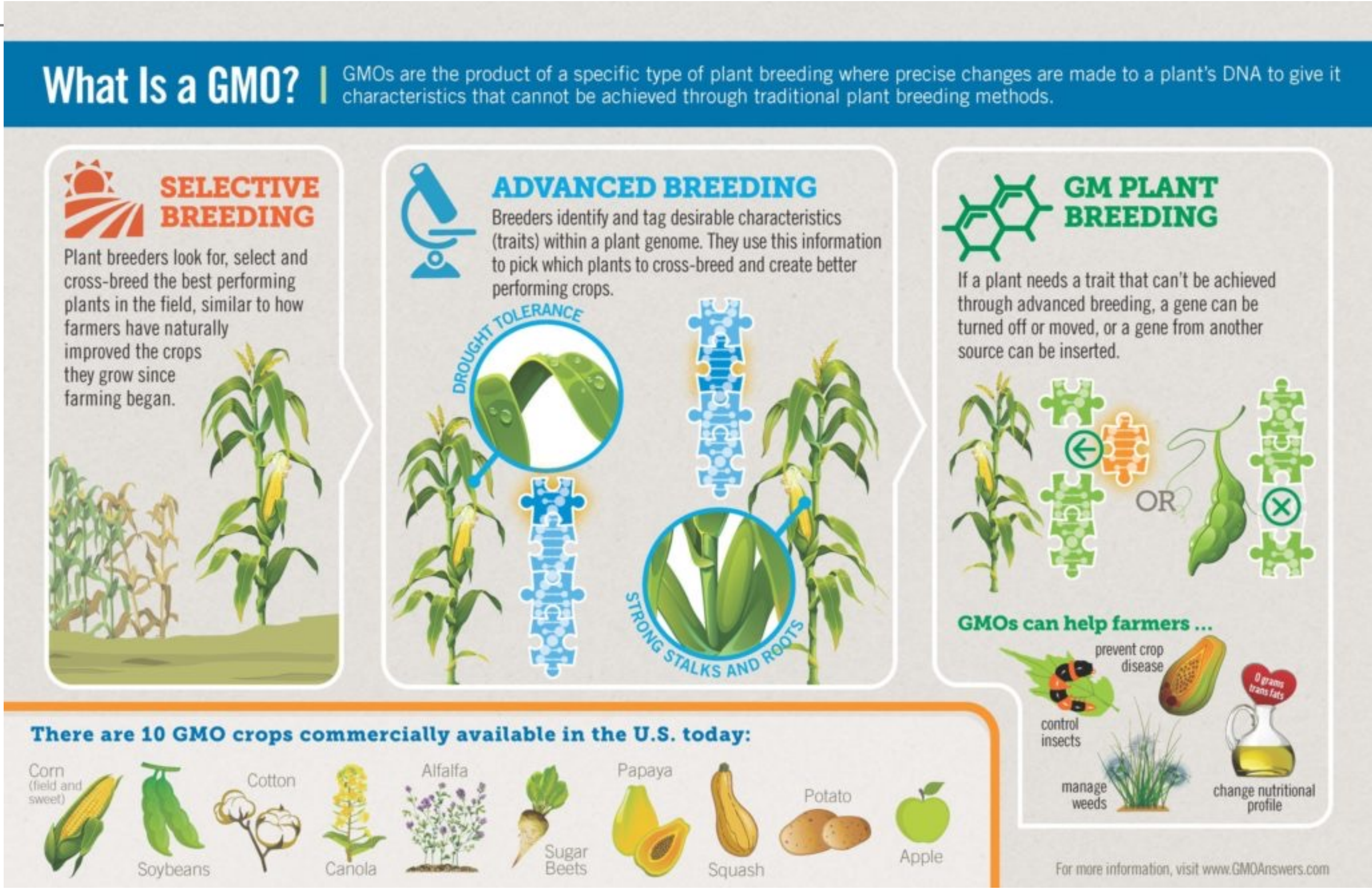
Pasta, bread and cookies, made with mixed flours containing ancient wheat species and other cereals.

→ need of analytical methods to determine **authenticity** of these products.

Tubulin-based polymorphism (TBP)

→ discriminate wheat and spelt (*T. spelta*), emmer (*T. dicoccum*), and einkorn (*T. monococcum*)

Sensitivity of 0.5–1% to authenticate the composition of food sample and detect possible adulterations.


What Is a GMO?
GMOs are the product of a specific type of plant breeding where precise changes are made to a plant's DNA to give it characteristics that cannot be achieved through traditional plant breeding methods.
SELECTIVE BREEDING
Plant breeders look for, select and cross-breed the best performing plants in the field, similar to how farmers have naturally improved the crops they grow since farming began.
ADVANCED BREEDING
Breeders identify and tag desirable characteristics (traits) within a plant genome. They use this information to pick which plants to cross-breed and create better performing crops.
DROUGHT TOLERANCE
STRONG STALKS AND ROOTS
GM PLANT BREEDING
If a plant needs a trait that can't be achieved through advanced breeding, a gene can be turned off or moved, or a gene from another source can be inserted.
OR
GMOs can help farmers ...
prevent crop disease
control insects
manage weeds
change nutritional profile
0 grams trans fats
There are 10 GMO crops commercially available in the U.S. today:
Corn (field and sweet)
Soybeans
Cotton
Canola
Alfalfa
Sugar Beets
Papaya
Squash
Potato
Apple
For more information, visit www.GMOAnswers.com

Video - Genetic Engineering & Our Food

Are GMOs Good or Bad?

https://www.youtube.com/watch?v=7TmcXYp8xu4

Arroz dourado

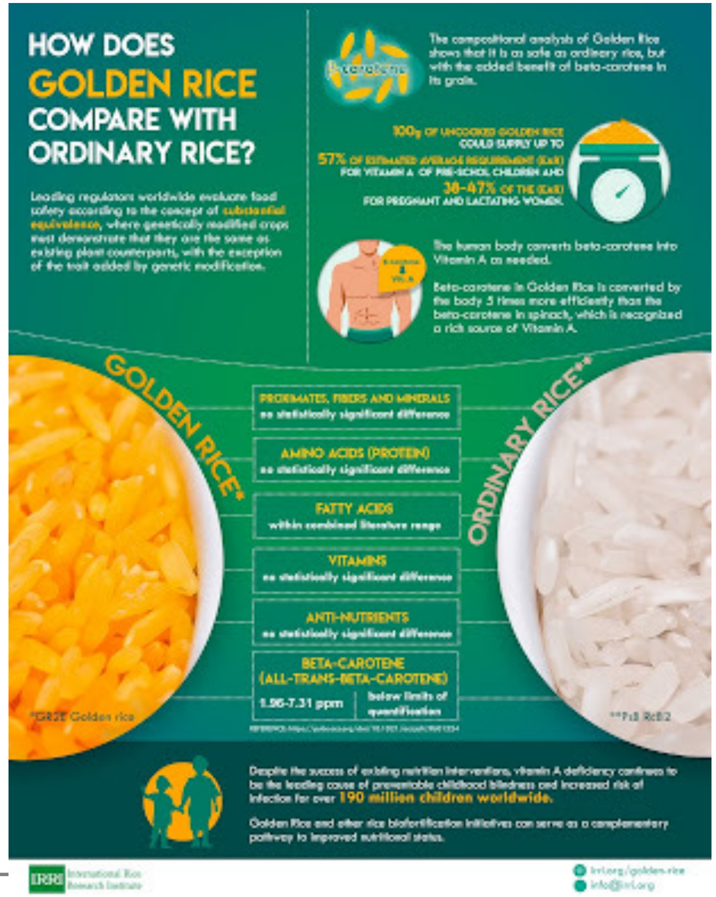
Estima-se que um milhão de crianças morre todos os anos com **deficiência em vitamina A** na Ásia onde a base da alimentação é o **arroz**.

Nos **anos 80** um cientista Suiço teve a ideia de produzir um arroz capaz de sintetizar **beta-caroteno** (precursor da vitamina A).

Em **1999** foi anunciado a primeira planta de arroz com uma coloração amarelada nos grãos.

Mesmo com esta causa estes cientistas viram-se envolvidos em várias polémicas tanto éticas como morais → 20 anos para ser aprovado.

O primeiro País a autorizar a sua plantação foi o Bangladesh, posteriormente as Filipinas



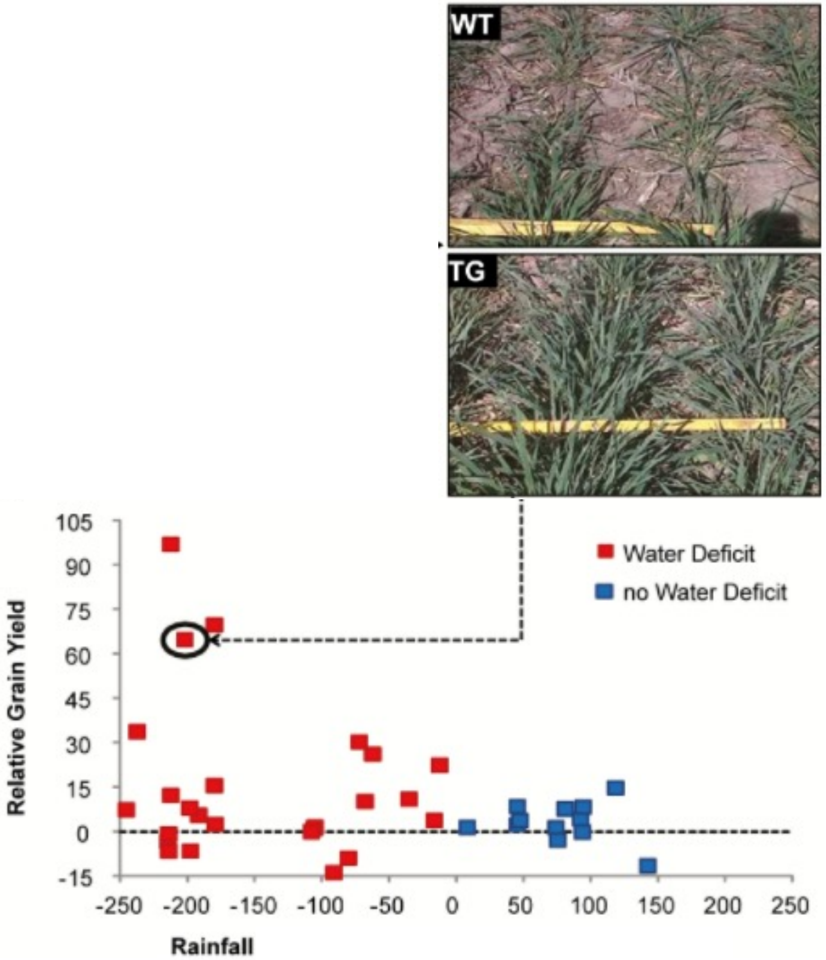
OGM Wheat IND-ØØ412-7
Drought tolerance

Drought is the major environmental stress affecting crop production.

HaHB4 (*Helianthus annuus* homeobox 4) gene from sunflower encodes for a transcription factor involved in tolerance to environmental stress.

HaHB4 was introduced in wheat IND-ØØ412-7 (**HB4 wheat**)

→ higher yield in environments with low productivity potential.



2019 Gonzalez et al - Field-grown transgenic wheat expressing the sunflower gene HaHB4 significantly outyields the wild type

OGM Wheat IND-ØØ412-7
Grain composition

Compositional analysis of IND-ØØ412-7 wheat

including 41 nutrients and 2 anti-nutrients for grain and 10 nutrients in forage

→IND-ØØ412-7 compositionally equivalent to non-transgenic wheat

Table 1 Proximates, starch, fiber, minerals and vitamins of grain from drought tolerant IND-ØØ412-7 wheat

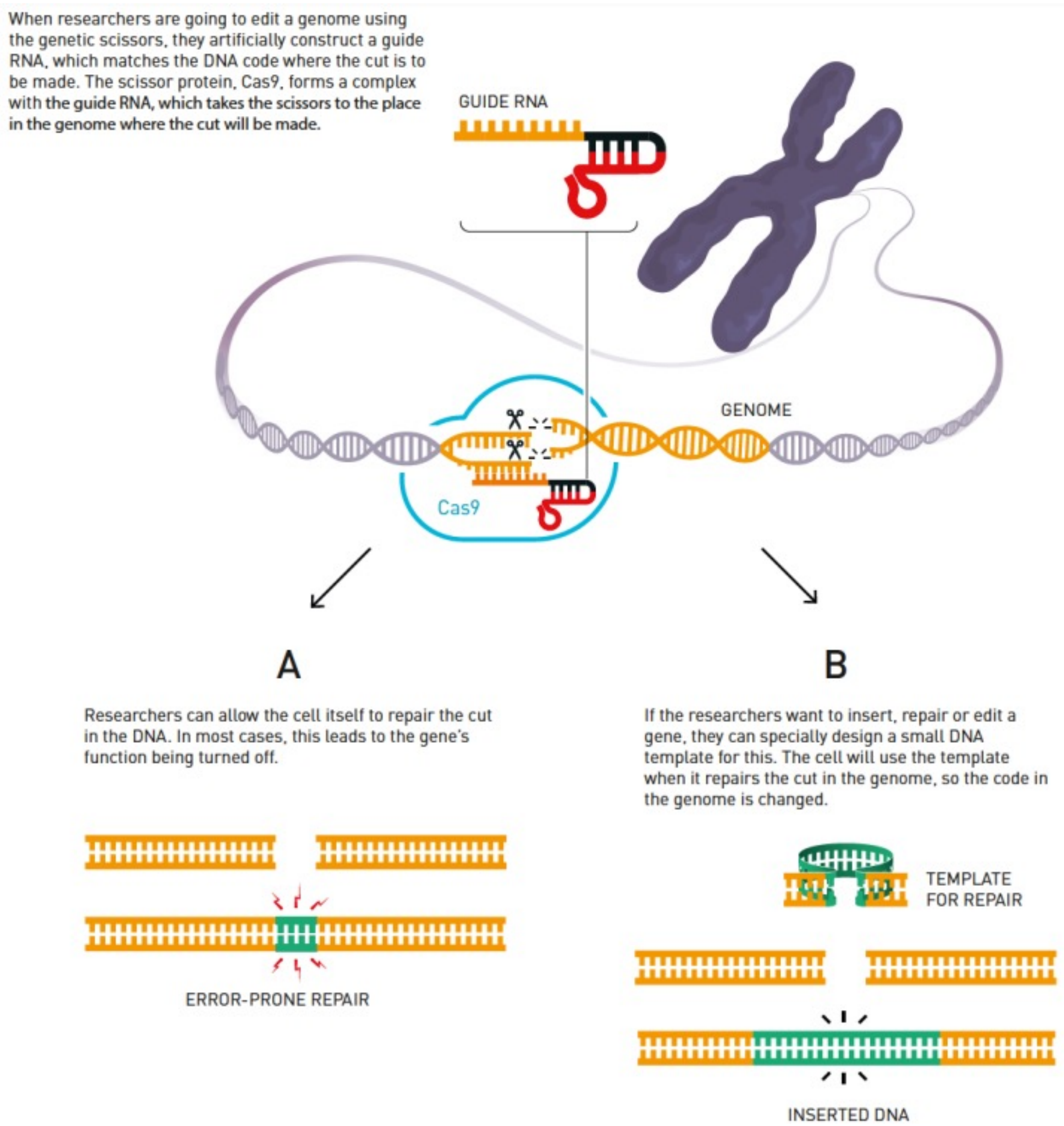
Component[a]	IND-ØØ412-7 mean (SE) (range)	Cadenza mean (SE) (range)	Commercial references range[b]	Literature range[c]
Ash	2.37 (0.09) (1.37–2.90)	2.32 (0.07) (1.69–2.79)	1.91–2.09	1.2–3.0
Carbohydrates	65.4 (0.0) (62.5–70.2)	65.8 (0.48) (63.0–70.3)	65.4–67.5	65.4–78.0
Moisture	13.09 (0.12) (12.14–14.75)	12.99 (0.16) (11.83–14.63)	13.99–14.30	8.0–18.0
Protein	16.2 (0.4) (12.3–18.4)	15.9 (0.3) (13.1–18.7)	14.2–15.2	10.0–16.0
Total fat	2.3 (0.0) (1.8–2.6)	2.2 (0.1) (1.6–2.7)	2.1–2.3	1.5–2.0
Starch	63.7 (0.5) (60.8–68.6)	63.7 (0.4) (61.1–69.3)	63.6–66.0	59–72
Dietary fiber	13.8 (0.2) (12.0–15.5)	13.9 (0.2) (11.6–16.0)	14.0–15.3	11.0–14.6
Calcium	461 (12) (373–573)	458 (12) (374–548)	441–501	250–538[d]
Iron	49 (2) (31–65)	50 (2) (30–76)	38–43	33–79[d]
Phosphorus	4912 (167) (3194–6146)	4961 (160) (3466–6061)	3970–4534	3320–5160[d]
Selenium	0.55 (0.03) (0.35–0.78)	0.55 (0.03) (0.37–0.82)	0.53–0.58	0.04–0.71[d]
Zinc	42 (2)* (22–63)	46 (2) (28–56)	32–35	24–47[d]
Thiamine	4.0 (0.1) (3.1–4.7)	4.1 (0.1) (3.2–5.0)	4.0–4.3	1.3–9.9
Riboflavin	0.43 (0.03) (0.25–0.81)	0.40 (0.02) (0.25–0.62)	0.48–0.66	0.6–3.1
Niacin	60.4 (2.2) (45.7–83.8)	58.8 (1.8) (46.7–80.8)	57.9–68.0	22.0–111.0
Pyridoxine	4.0 (0.1) (3.3–4.9)	4.1 (0.1) (3.3–4.8)	3.9–4.2	0.9–7.9
Folic acid	0.29 (0.01)* (0.17–0.38)	0.31 (0.01) (0.16–0.40)	0.27–0.33	0.2–0.9
α-Tocopherol	10.7 (0.4) (6.5–14.0)	10.6 (0.3) (7.7–13.7)	8.4–9.5	9–18

2019 Ayala et al - Compositional equivalence of event IND-ØØ412-7 to non-transgenic wheat

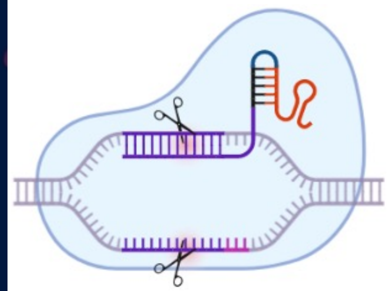
CRISPR/Cas9
genetic scissors

→ Genes turned off

→ Gene insert,
repair or edit

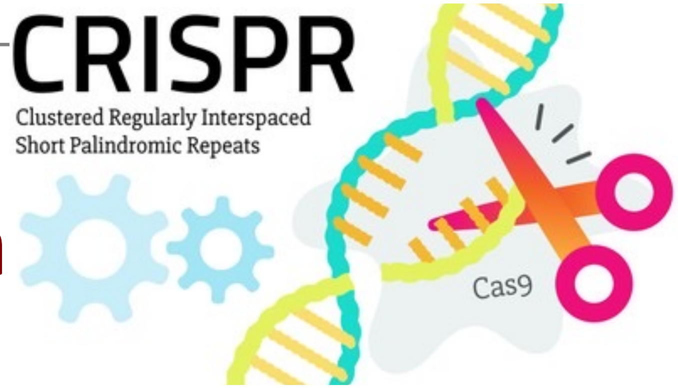


Video - CRISPR/Cas9 Genetic engineering will change everything



https://innovativegenomics.org/multimedia-library/kurzgesagt-video-animates-crispr/

CRISPR/Cas9
for **crop improvemen**



- **Gene editing** using CRISPR/Cas

Can play a major role in ensuring food security developing

- resilient commercial crops
- improved yield
- improved nutritional value

→ mutation in genes and regulatory regions induce variable phenotypes

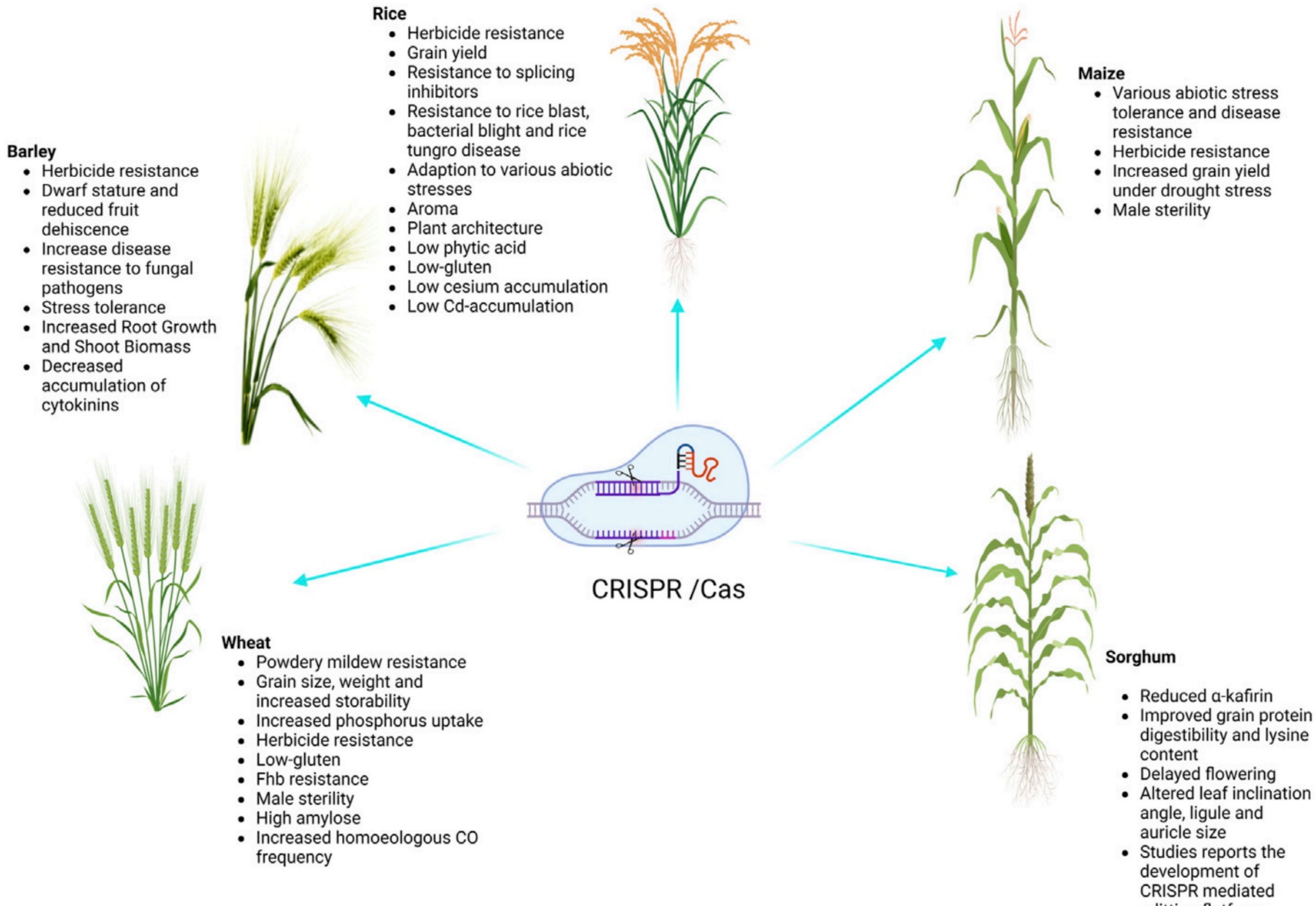
breeding program

CRISPR/Cas gene editing in cereal crops

Rice		Golden rice 1 & golden rice 2	Targeted gene insertion	High β-carotene in grains	Dong et al. (2020)
Rice		*Cytochrome P450s* and *OsBADH2*	Loss of function	Improved fragrance in grains	Usman et al. (2020)
Maize		*ZmSH2 & WX*	Loss of function	Super sweet and waxy corn	Dong et al. (2019)
Maize		Multiple genes	Loss of function	Characterized novel genes for agronomic and nutritional importance in maize	Liu et al. (2020)
Wheat		*TaGW7*	Loss of function	Enhanced weight and shape of wheat grain	Wang, Pan, et al. (2019)
Wheat		*Taα-gliadin*	Loss of function	Less gluten content in wheat grain	Sanchez-Leon et al. (2018)
Barley		*Hv d-Hordein*	Loss of function	Increased starch content, amylose content, and beta-glucan content	Yang et al. (2020)

2020 Tiwai et al - Emerging tools and paradigm shift of gene editing in cereals, fruits, and horticultural crops for enhancing nutritional value and food security

Successful application of CRISPR/Cas genome editing in cereals



2023 Basu et al - A CRISPR way for accelerating cereal crop improvement: Progress and challenges