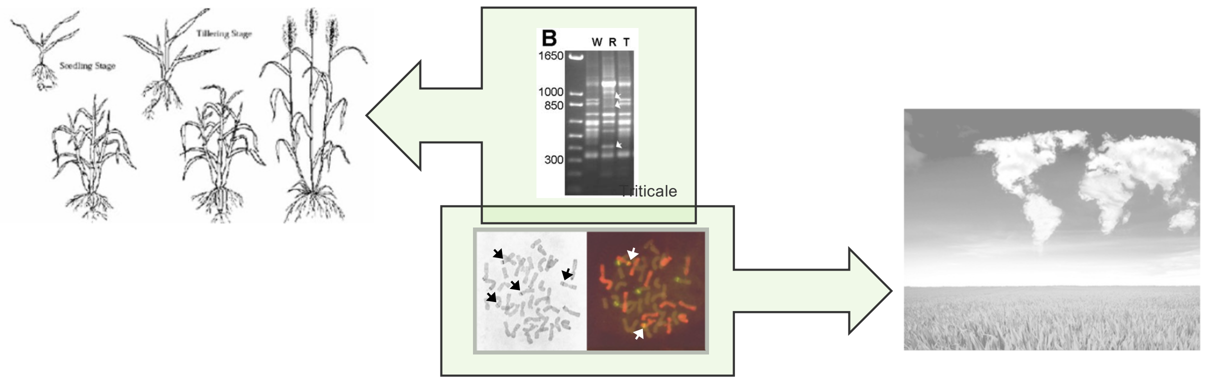
Mestrado em Engenharia Alimentar

Tecnologia dos Cereais

Cereal Evolution and Genomics

Manuela Gomes da Silva



Índice

Understanding **Cereal Genomics**

Cereals <u>economic and scientific</u> **importance**

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

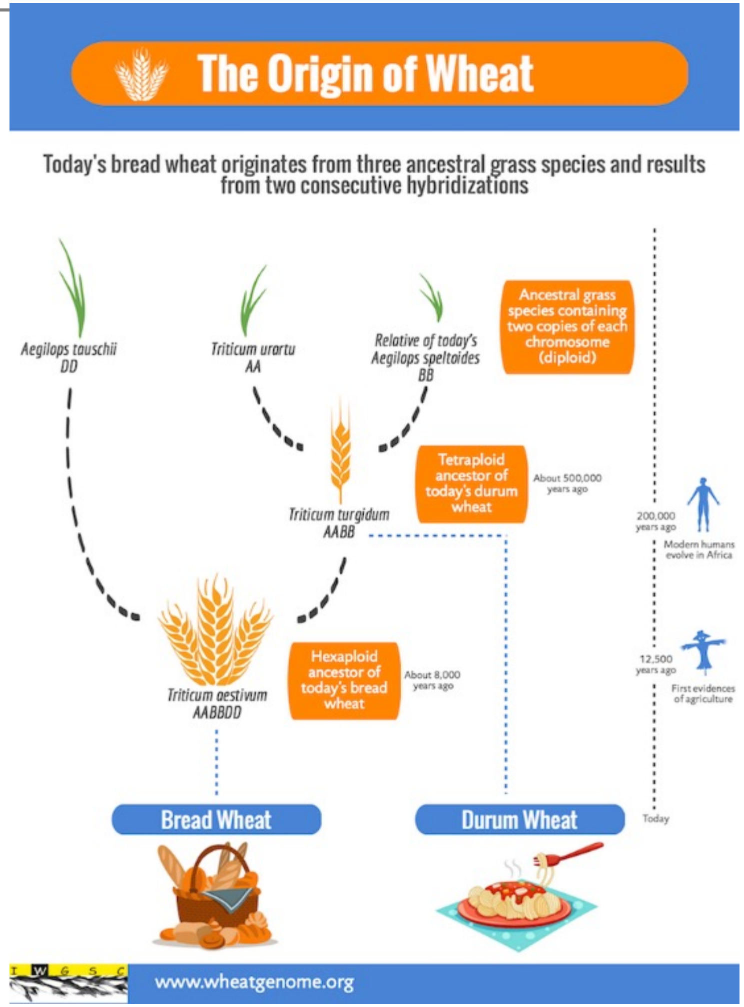
Relationships among morphologically <u>diverse cereals</u> from globally <u>geographic environments</u>

→ particularly attractive for <u>comparative studies</u> of **plant genome evolution**

Complete <u>genome sequence</u> of **rice** (2002)

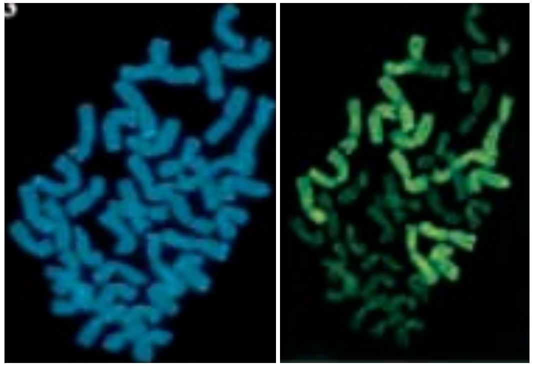
→ transition to high-throughput **genomics study** of many other cereals

… **wheat** sequenced 2018



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

Importance of **cytogenetics** and **molecular biology** for cereal study

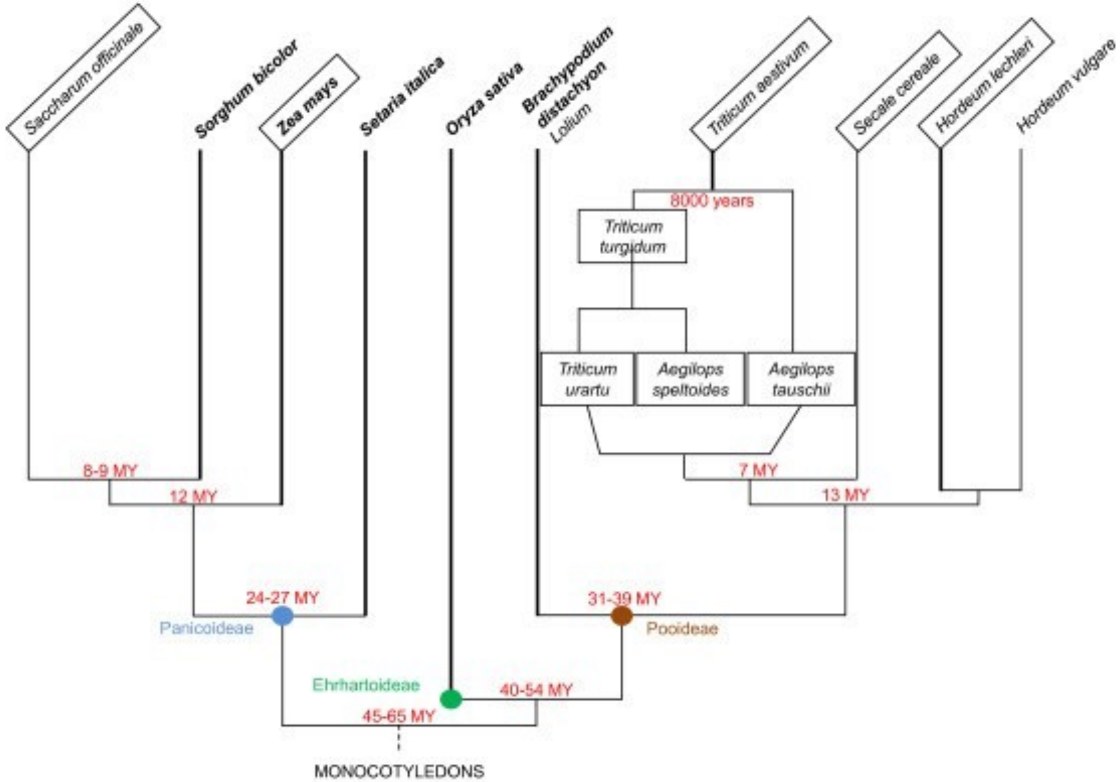


FISH of hexaploid triticale metaphase

→ to **understand** the

evolution of **cereal genomes**

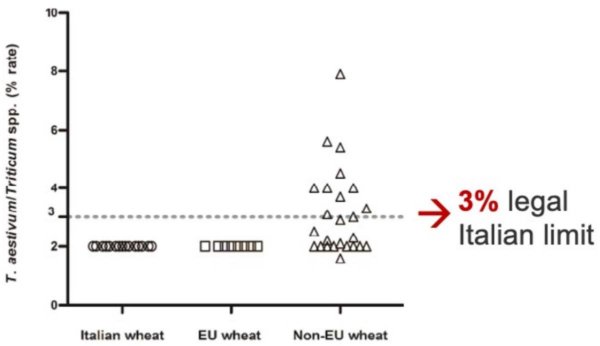
Design strategies for **Plant Breeding**



Poaceae phylogenomics based on a biosynthetic pathway

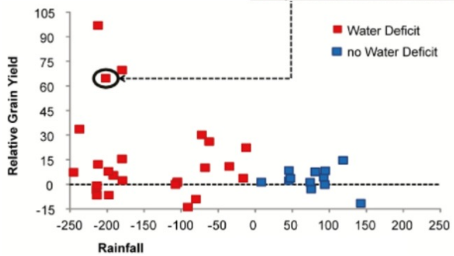
Dutartre et al 2012

Use of **Molecular Biology** tools

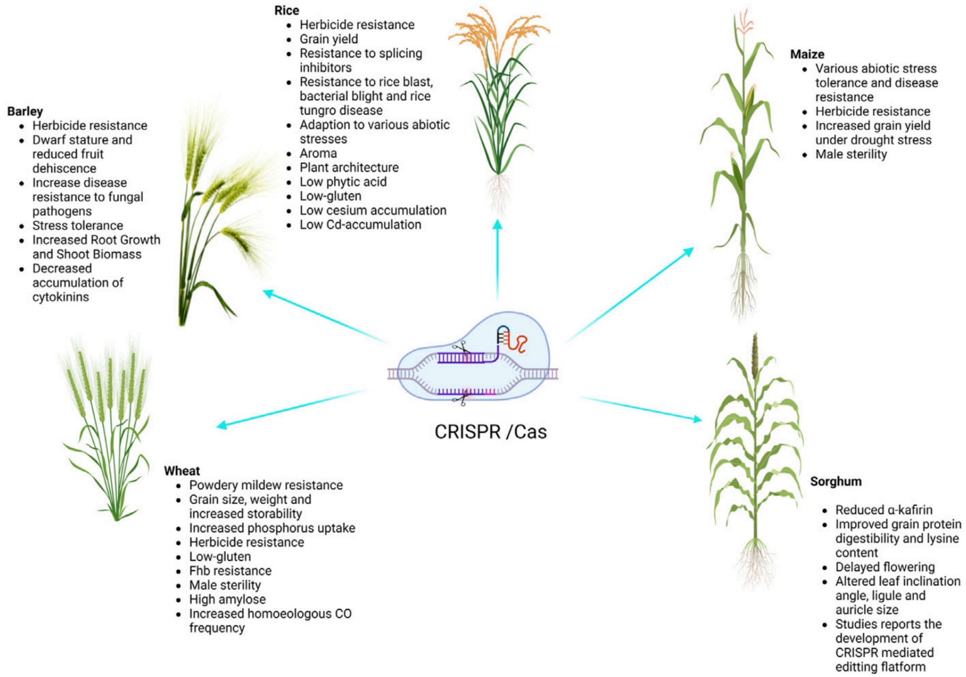


Quantification of *T. aestivum* in Italian, European Union (EU), and Non-EU semola

Italian pasta **certification**



OGM wheat drought tolerant



Successful applications of **CRISPR/Cas9**

Importance of **Cereal Biotechnology**

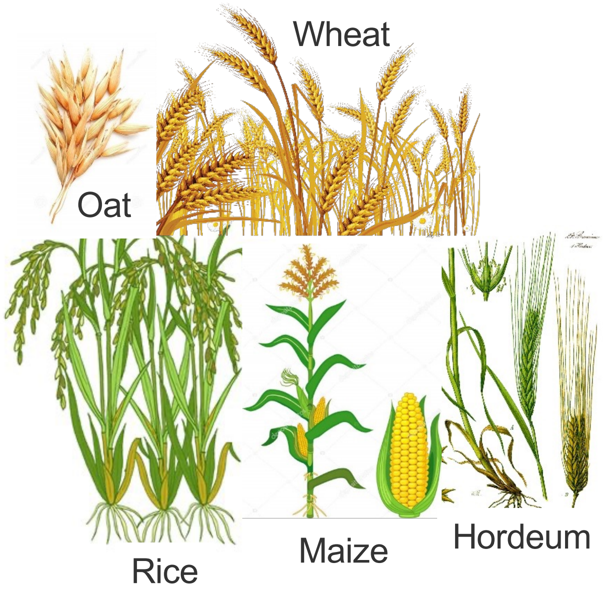
Polyploid plants - **Gramineae**

Many crop plants are polyploid

Potato, cotton, coffee, tobacco, banana…

A sample of agricultural products obtained from polyploid crops

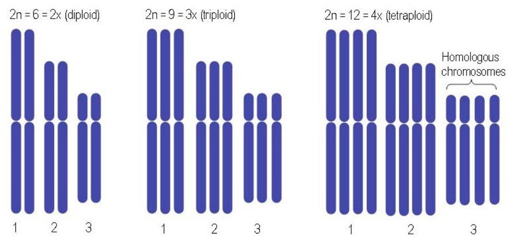
Polyploidy is particularly frequent in **Poaceae** family (**Gramineae**)



Leitch and Leitch, 2008

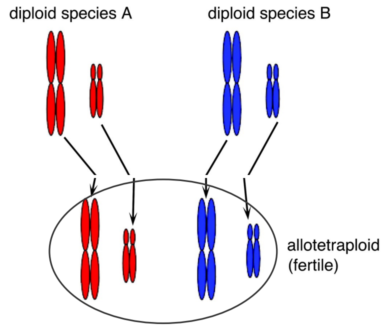
Polyploidy

- POLYPLOID - plants with <u>multiple copies</u> of a genome or with <u>different genomes</u>



- Autopolyploid ← **duplication** of one genome

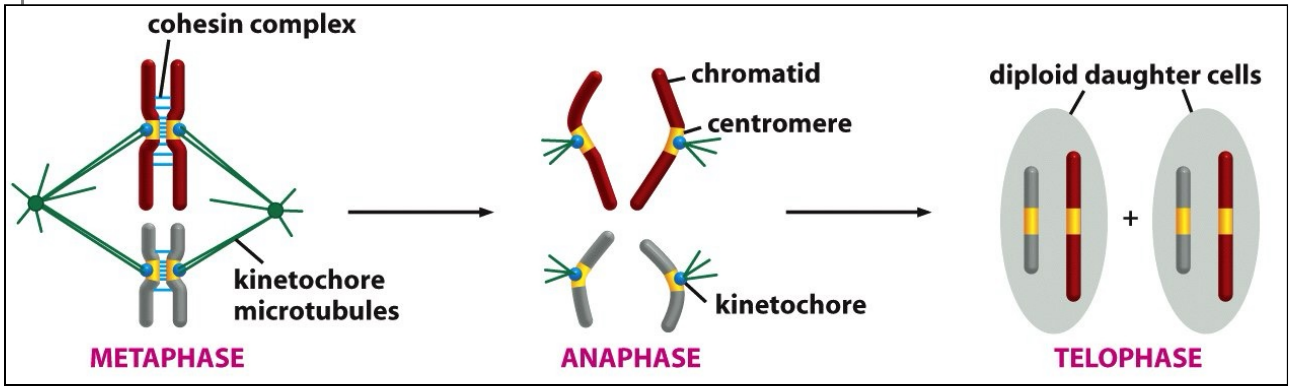
- Allopolyploid ← **combination** of 2 or more different genomes



How do polyploid plants arise??

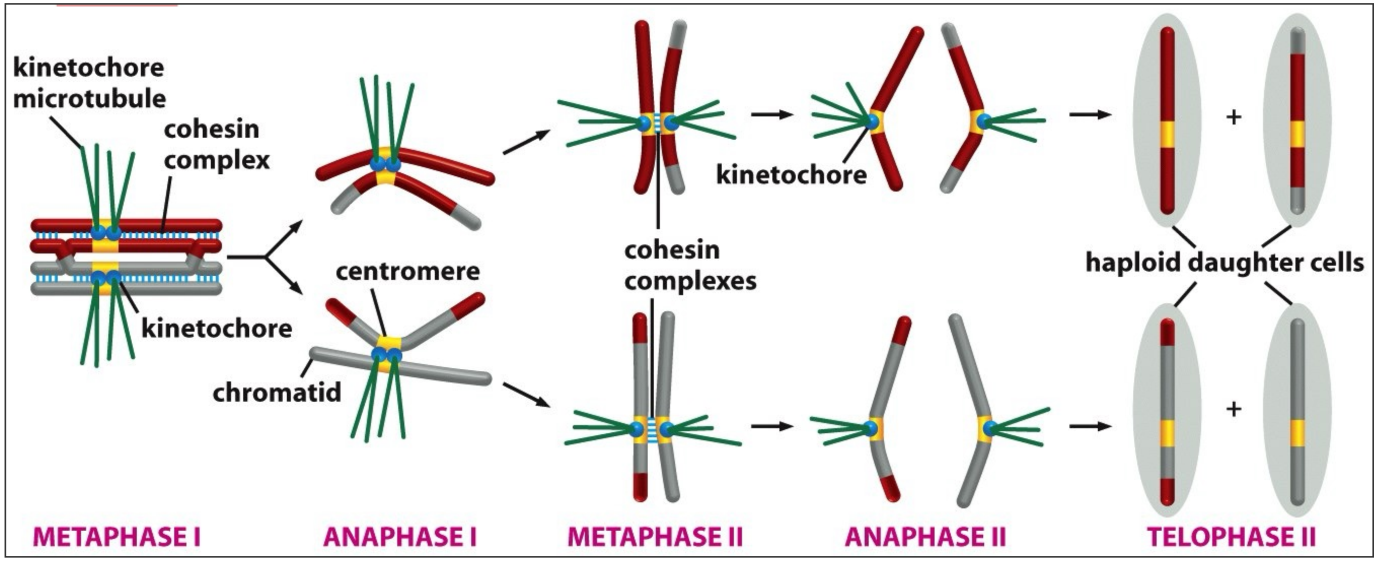
→ **Mitotic or Meiotic errors**

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



MITOSE

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



MEIOSE

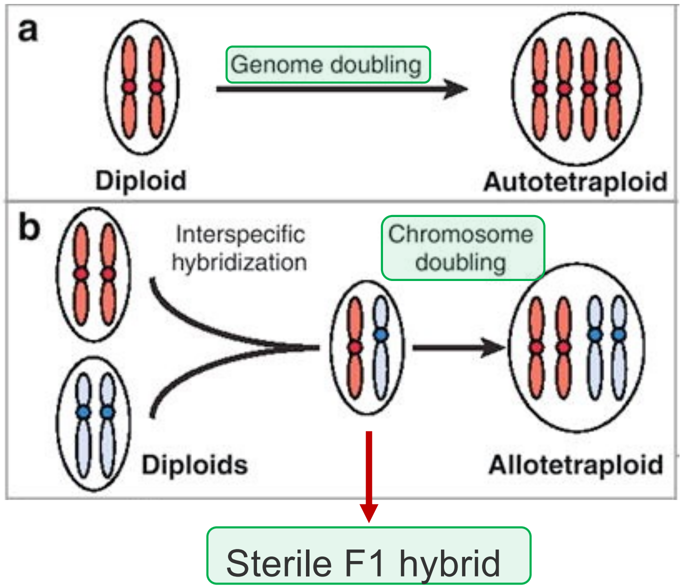
1 duplicação - 2 divisões
→ 4 células haploides (n)

Polyploidy origin - Mitotic anomalies

→ **somatic chromosome doubling**

Diploid species doubling → **autopolyploid**

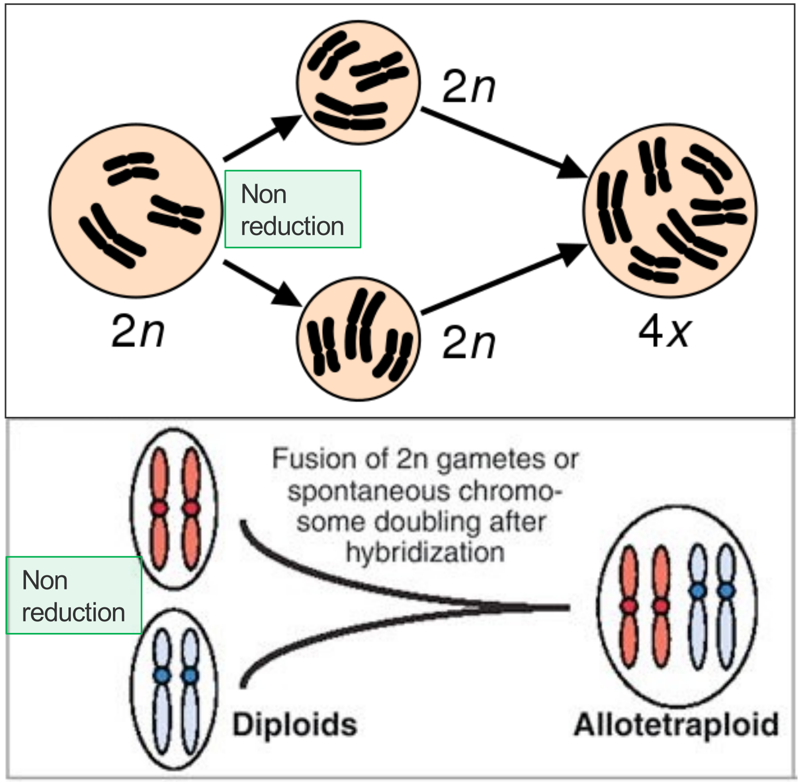
F1 hybrid doubling → **allopolyploid**



(a) Formation of an **autotetraploid** by **chromosome doubling**

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

Polyploidy origin - **Meiotic** anomalies → 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**

Cereals

"Two-step" model

interspecific hybridization

chromosome doubling of the F1 hybrid

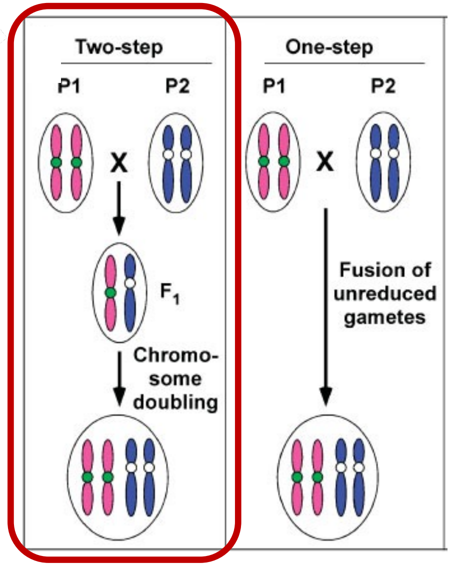
→ most common process

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

(chemical chromosome-doubling)

"One-step" model

fertilization of unreduced gametes from different diploid species



Chen, 2007

Atividade – 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’’

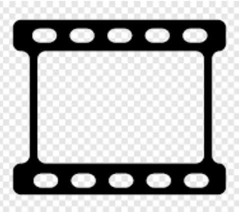
https://www.youtube.com/watch?v=zrKdz93WlVk&ab_channel=AmoebaSisters

2- Polyploidy leads to speciation (IB Biology) 4’48’’

https://www.youtube.com/watch?v=odL631acMC0&ab_channel=AlexLee

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

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



4- Is GMO wheat the answer to food security? - Cristobal Uauy 5’03’’

https://www.youtube.com/watch?v=g0bOBkSnNkQ&ab_channel=GatsbyPlantScienceEducationProgramme

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

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

6 - Sanger Sequencing of DNA 3’39’’

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

What **plants** do better than animals?

In terms of reproduction, plants have more options than animals:

→ sexual and asexual

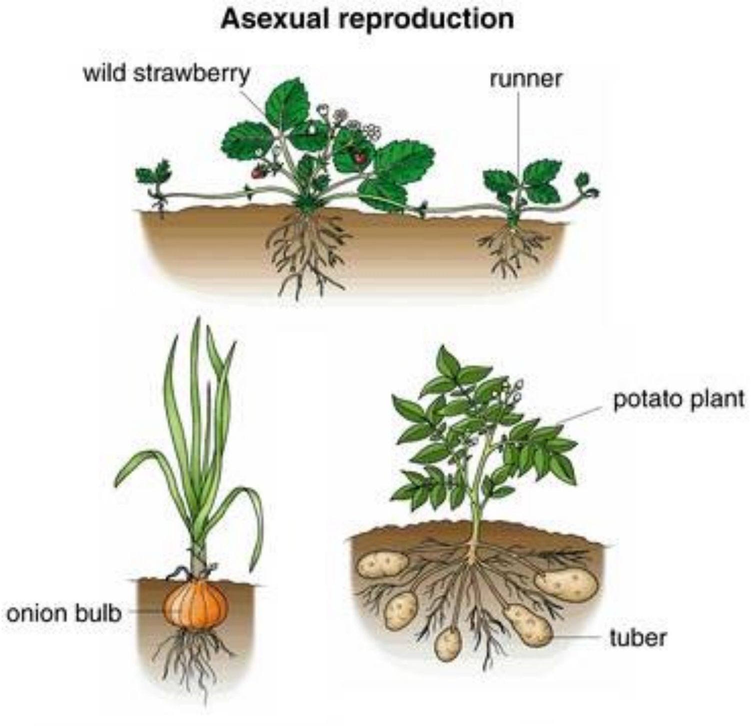
- Hybridization
  - Vegetative reproduction
- Polyploidy

Species A X Species B

↓

Sterile hybrid

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



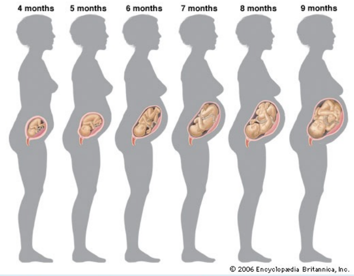
Why Polyploidy is less common in **animals**?

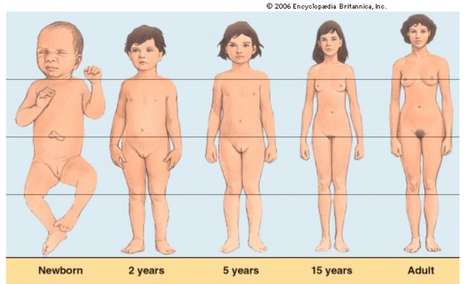
ANIMALS

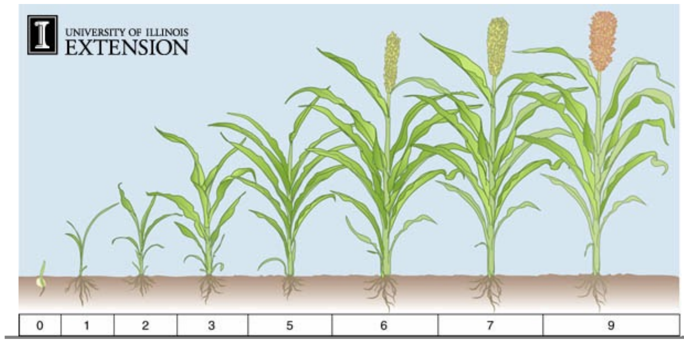
Chromosomally determined **sex** (polyploidy interference)

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

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







PLANTS

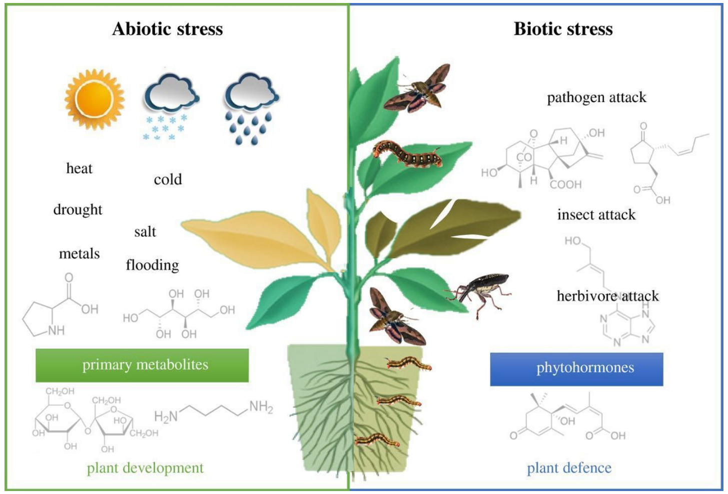
Meristematic tissue throughout their lives and self-fertile

Advantages of Polyploidy

- **Higher diversity**
- Duplicated gene copies free to mutate

  → **evolve** to assume **new functions**

- Increased **adaptability** to a higher ecological range
- Enhanced abiotic and biotic **stress tolerance**
- **Larger** cells and organs

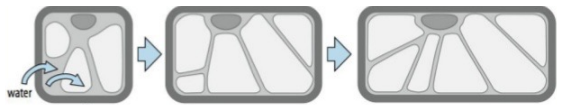


Polyploidy → Larger cells and organs

Diploid and **autotetra**ploid cultivars of
Lolium perenne and *L. multiflorum* (Gramineae)

- **Autopolyploid longer leaves**, longer mature cells (20x)
  (epidermal and mesophyl)
  ← **higher cell elongation rate**,
  not by a longer period of cell elongation

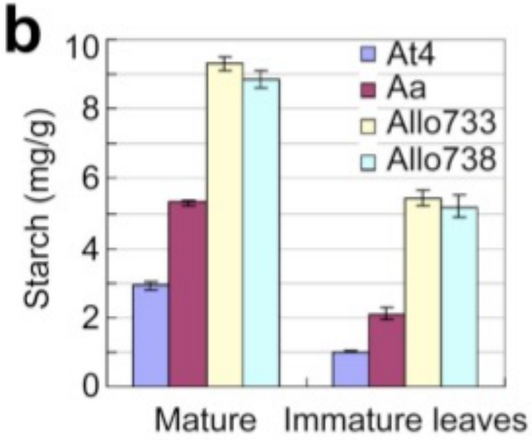
- No variation in cell division parameters
  (cell production rate and cell cycle time)

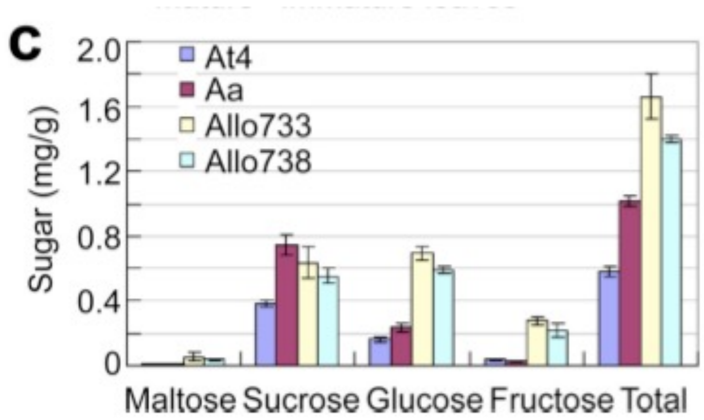


Sugiyama , 2005

Molecular basis for **polyploid vigor**

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





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

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

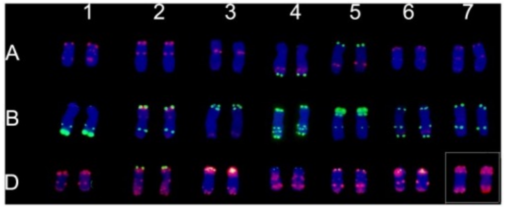
→ **growing larger** in comparison with their parents

Ni et al, 2009

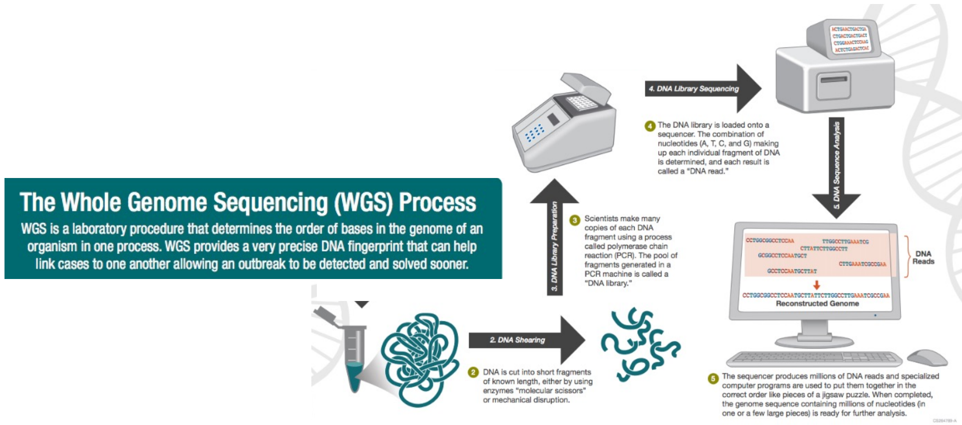
Polyploidy detection

- **Polyploid** species – detected by <u>cytogenetics</u>

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



- **Paleopolyploids** – detected by genome <u>sequencing</u>



Cereal genome **cytogenetics**

Metodologias citogenéticas

- Estabelecimento de **cariótipos**

  - **Técnicas de bandeamento**

    → Atividade

Cytogenetics

Cytogenetics = Cellular Genetics

- Branch of genetics that studies **cells** and especially **chromosomes**

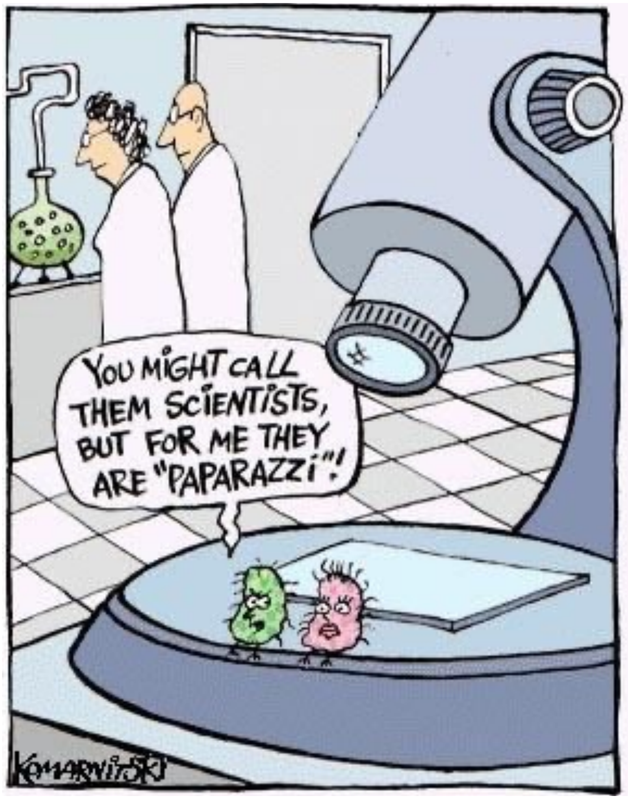
Cytogenetic tools

→ Conventional **karyotyping**

Chromosome banding

→ **Molecular cytogenetics**

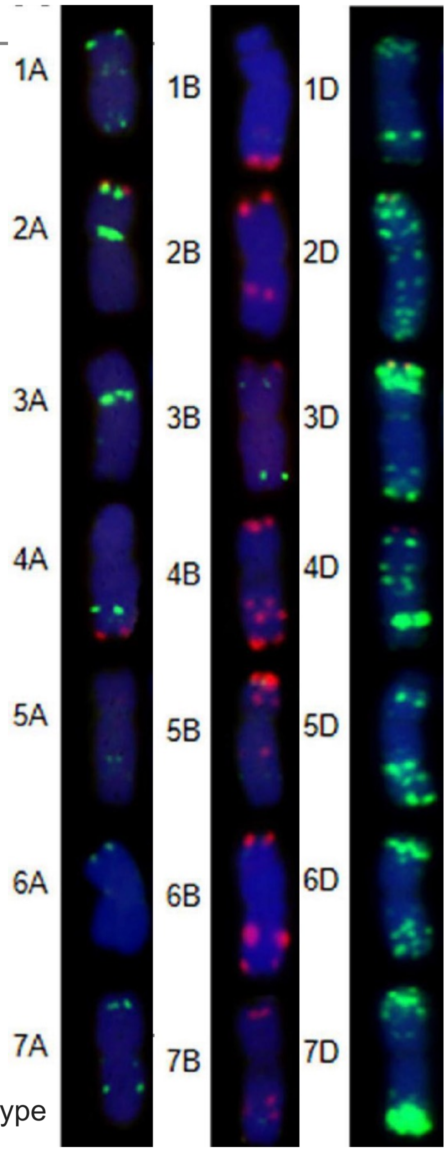
Fluorescence In Situ Hybridization (FISH)



Estudo de cariótipos

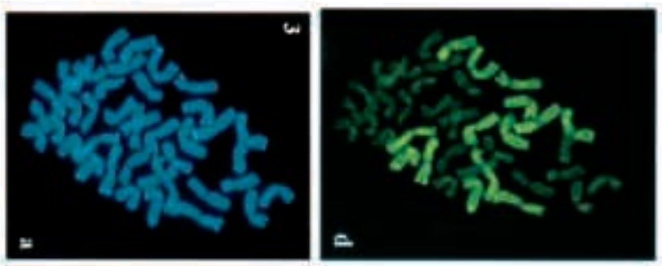
Fluorescence In Situ Hybridization (FISH)

- **bandeamento** para estabelecimento de cariótipos



Wheat FISH karyotype

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



Cromossomas de triticale

Verde - centeio

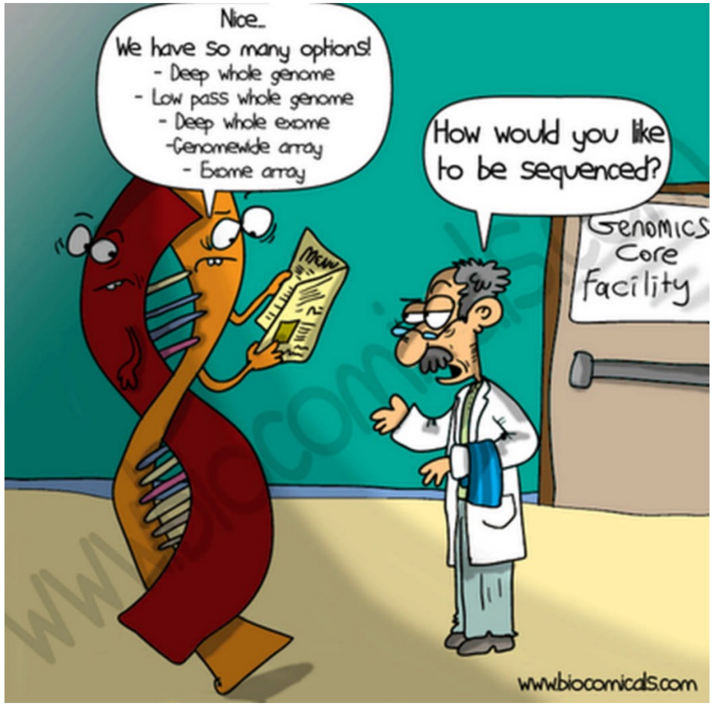
Azul – DNA corado com DAPI

Sequencing

- **"Sequencing"** – **order** of **nucleotides** in the **DNA** molecules

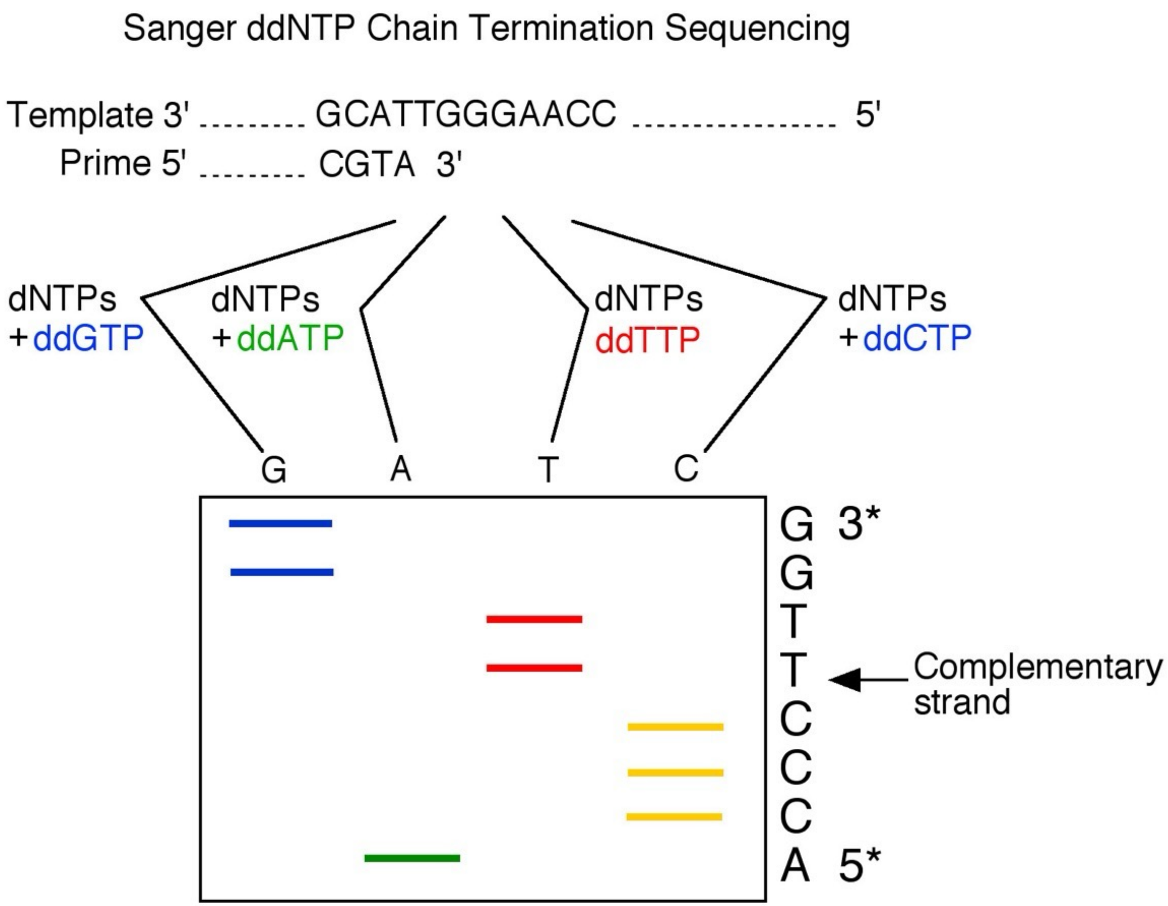
Nucleotides order → amino acids order → protein structure and function

- Changes in the DNA (correlated with changes in proteins)



→ Allowed the **identification on paleopolyploid species**

The Sanger method

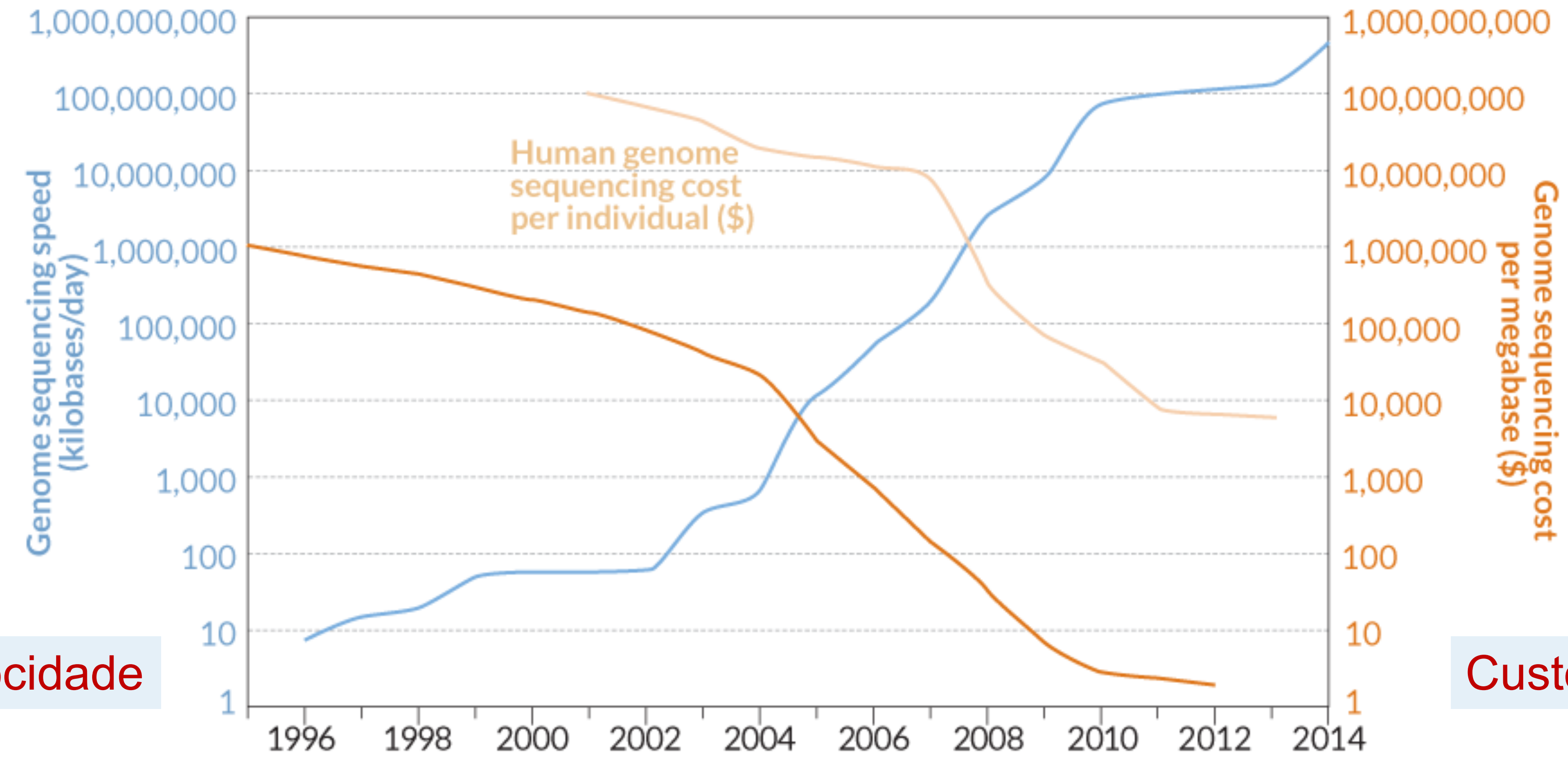


Sanger Sequencing

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



Velocidade

Custo

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

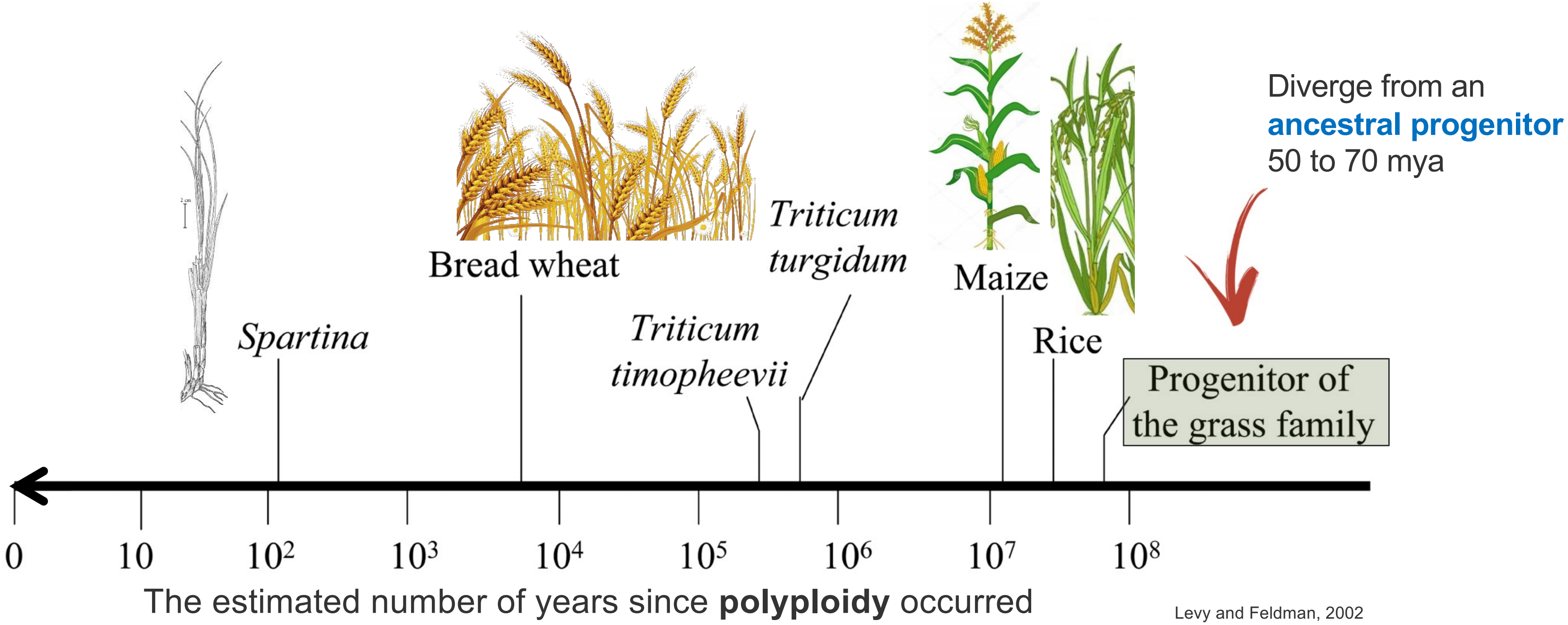
Gramineae polyploids

Poaceae (Gramineae) - 10,000 species, 600 to 700 genera

Common ancestral - 50 to 70 million years ago

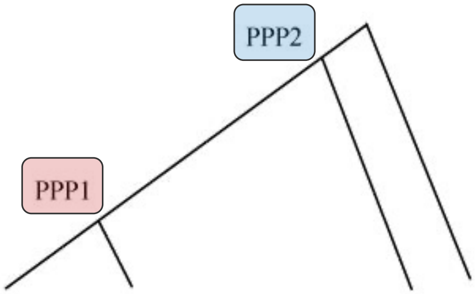
Polyploidy in grasses is an ongoing process

Cereal species **selection** - domestication and improvement



Levy and Feldman, 2002

Rice (*Oryza sativa*) – ancient polyploid



Rice genome analysis

PPP2 - <u>older</u> genome of **monocotdicot** ancestral

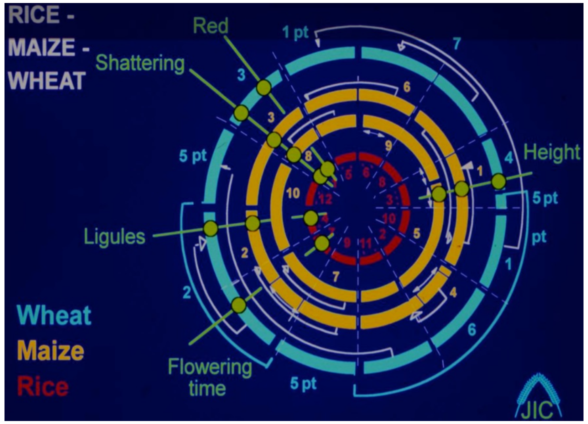
PPP1 - ancient genome duplication of all **cereals** ancestral

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

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

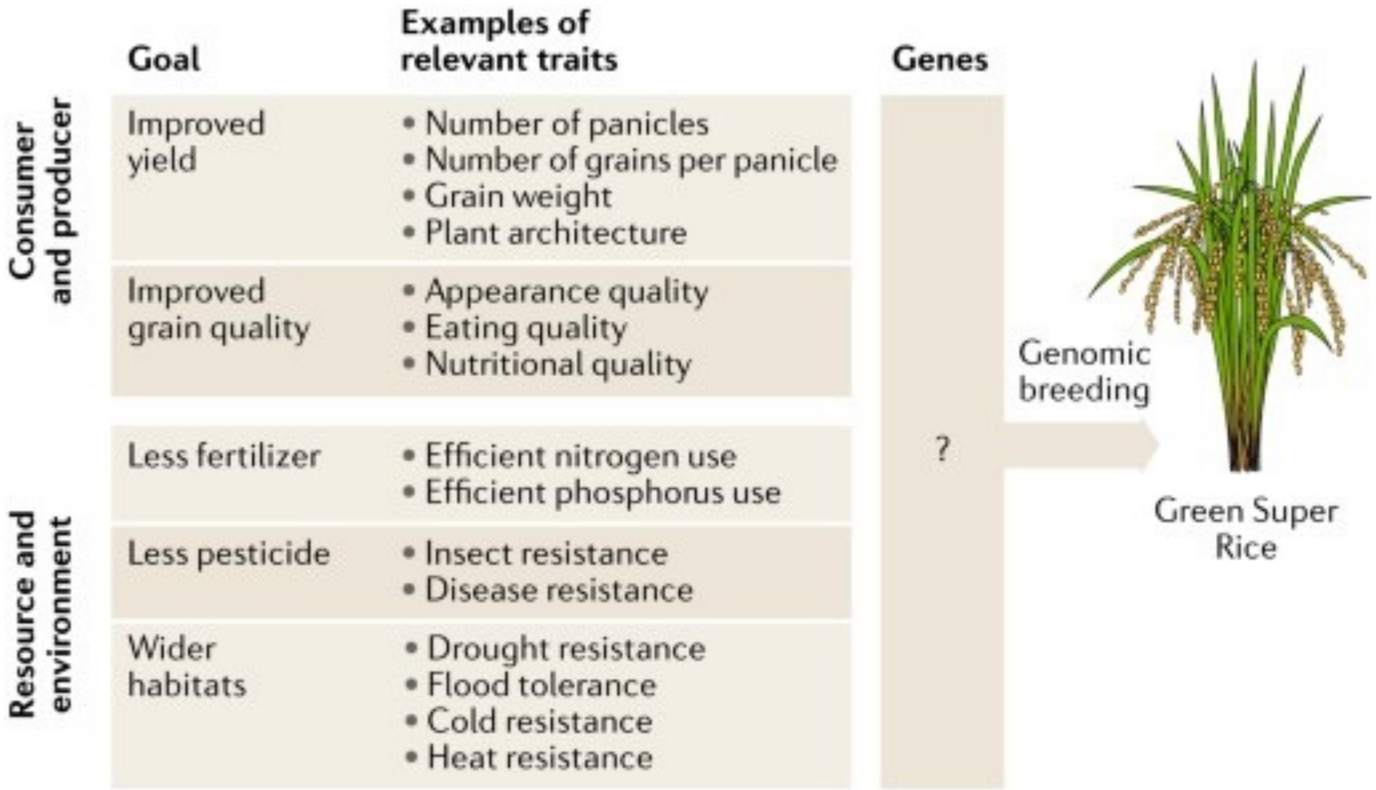
- Model cereal crop - **small genome** size (2n = 24)

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



Zhang et a 2005

Rice – from ancient polyploid to breeding

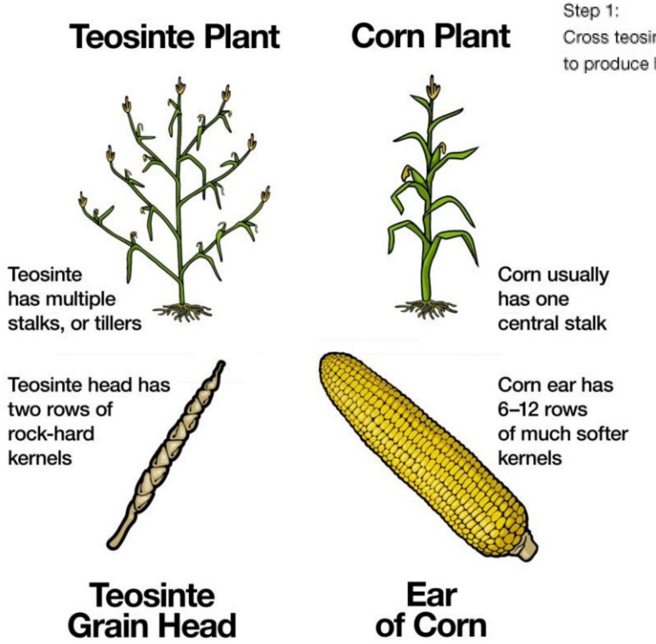


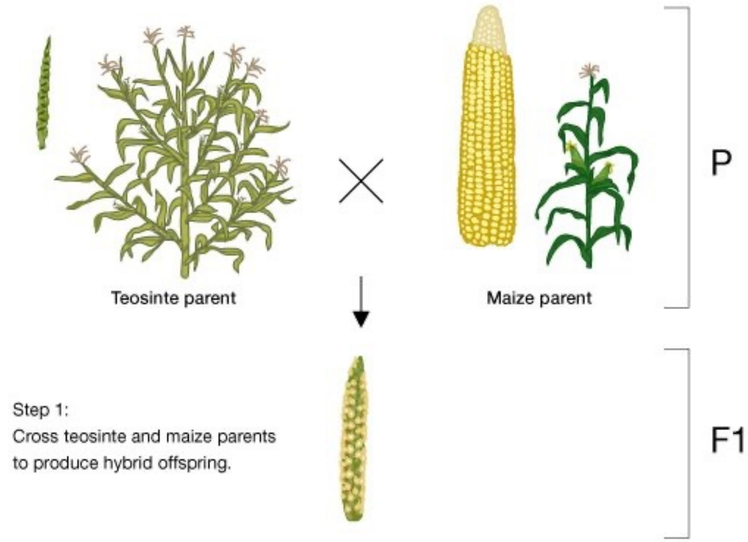
→ **Genetic variation** among <u>domesticated rice</u> species and their **wild relatives** has been investigated to identifying traits that can be **exploited for breed**

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**.

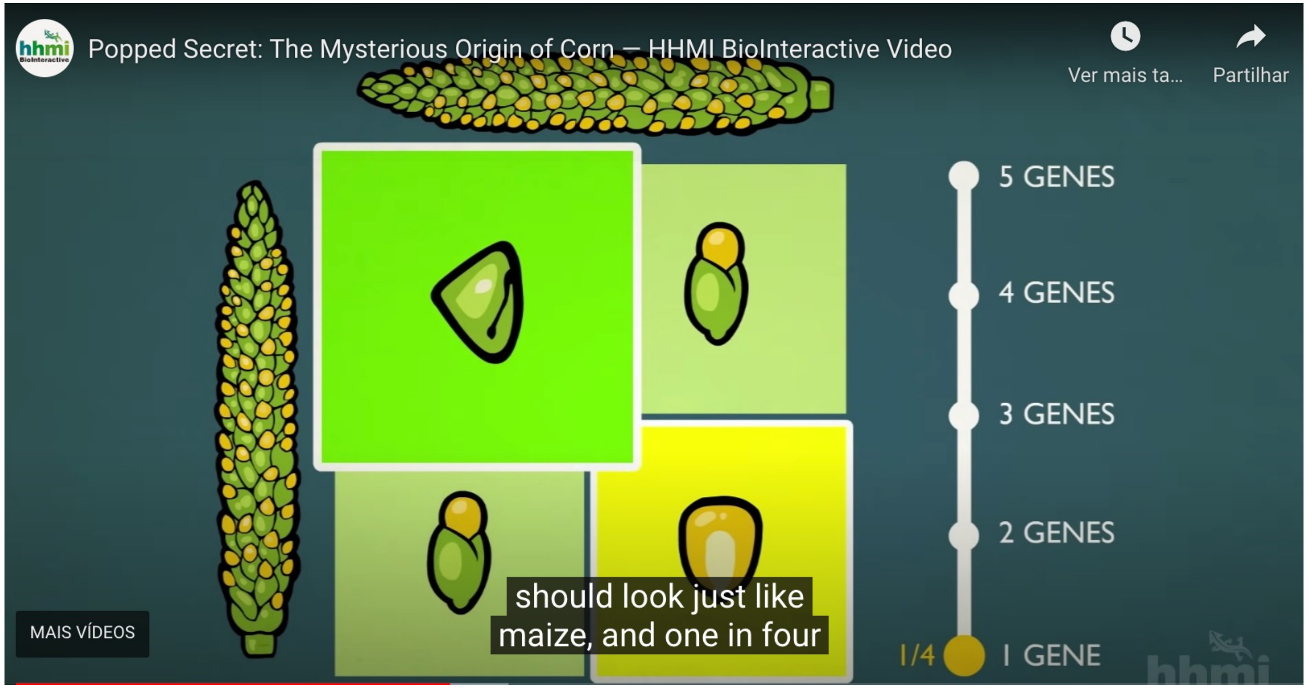




George Beadle 1930s - **teosinte-maize hybrids** → chromosomes are highly compatible.

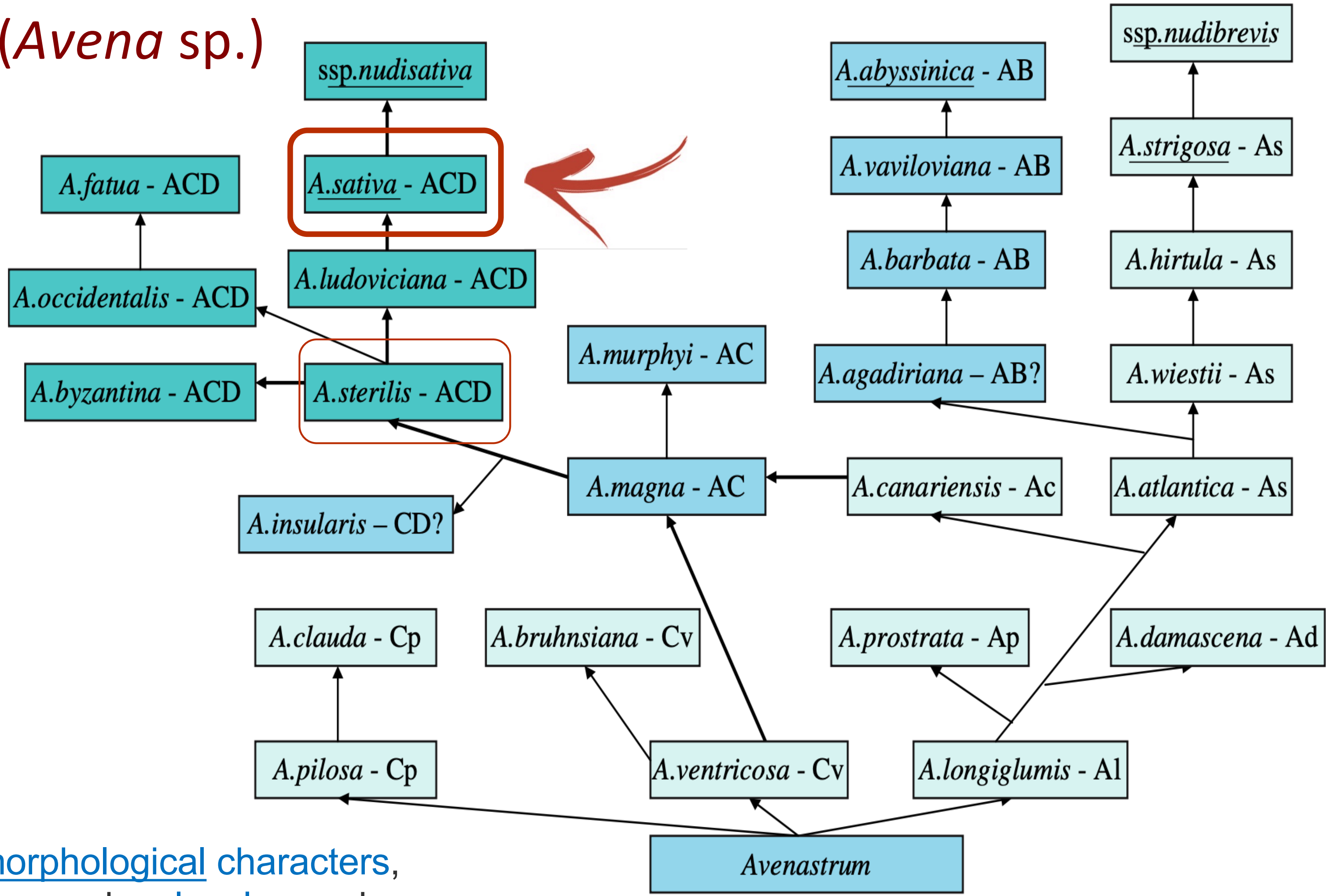
Calculated that only about **5 genes** were responsible for the notable differences between teosinte and a primitive strain of maize.

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



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

Oat evolution (*Avena* sp.)



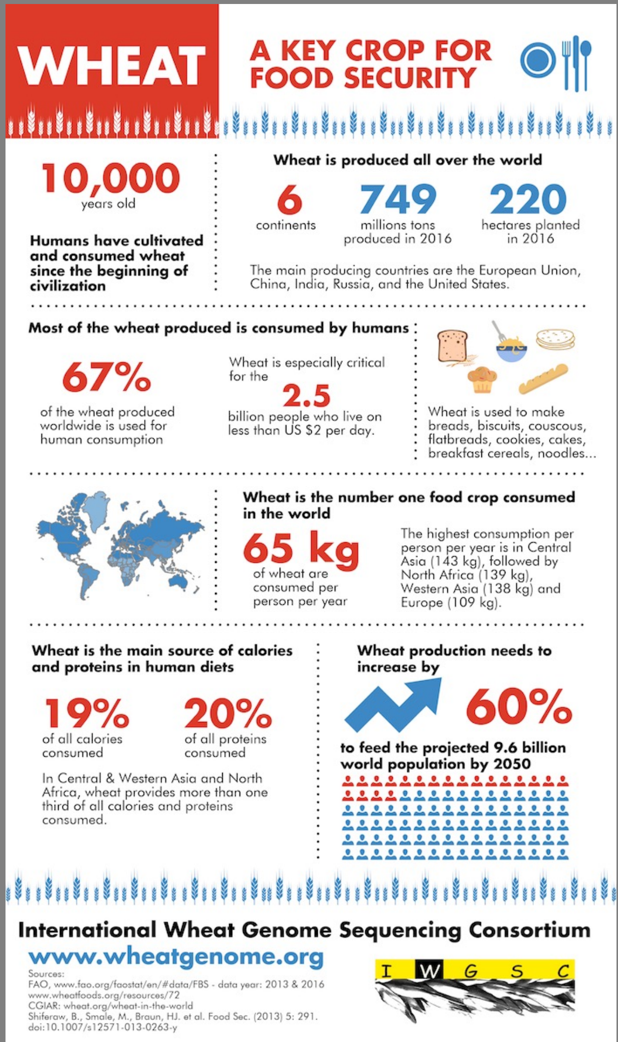
Study of major morphological characters, karyotype structure and molecular markers

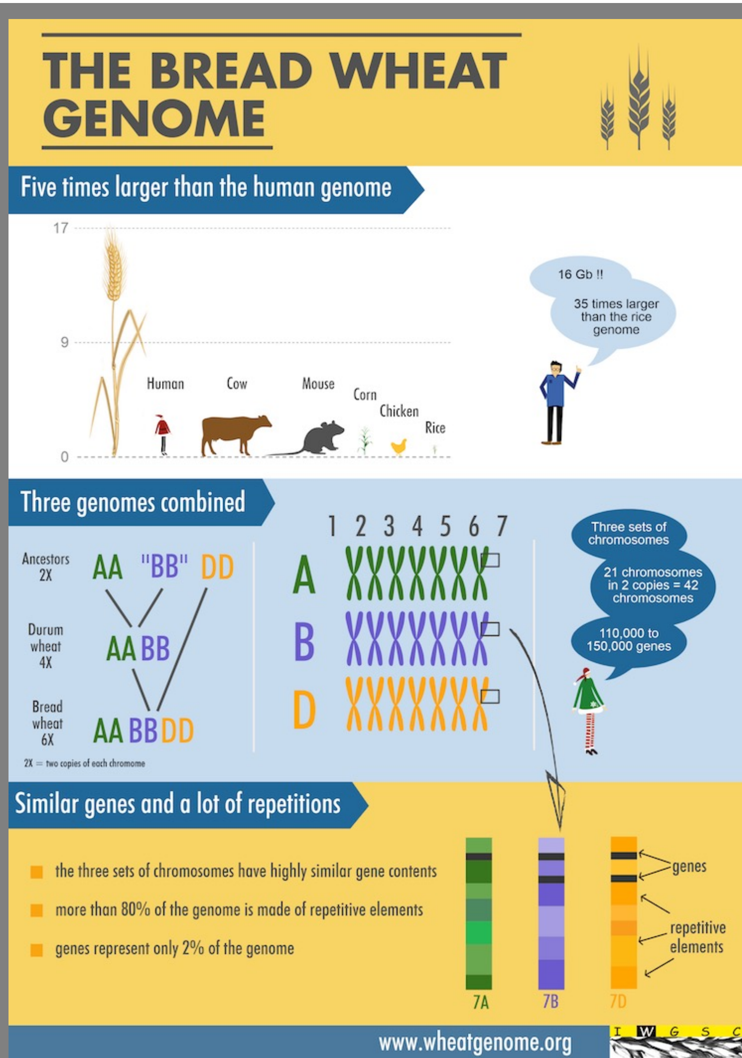
2008 Loskutov

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

Production must increase 60% until 2050

Human main source of **calories** and protein

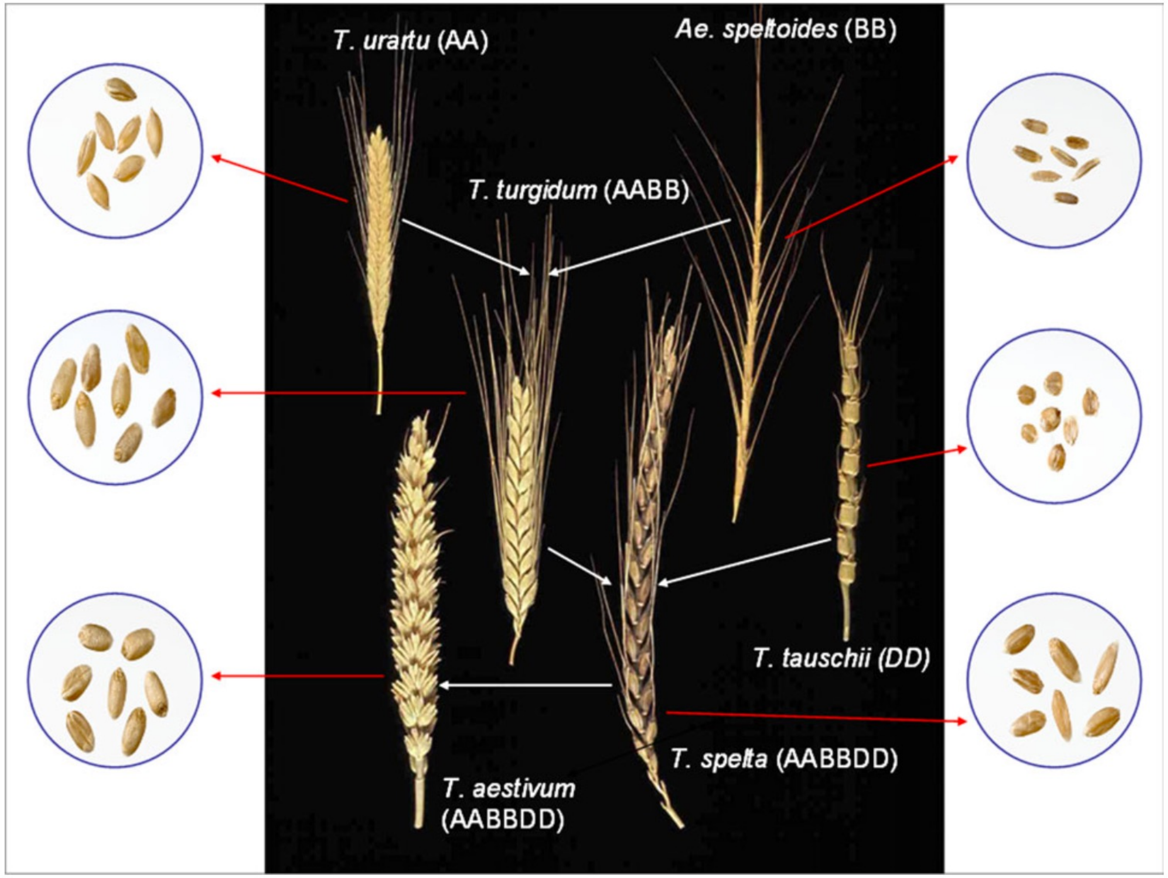




Wheat genome size **35x** rice genome

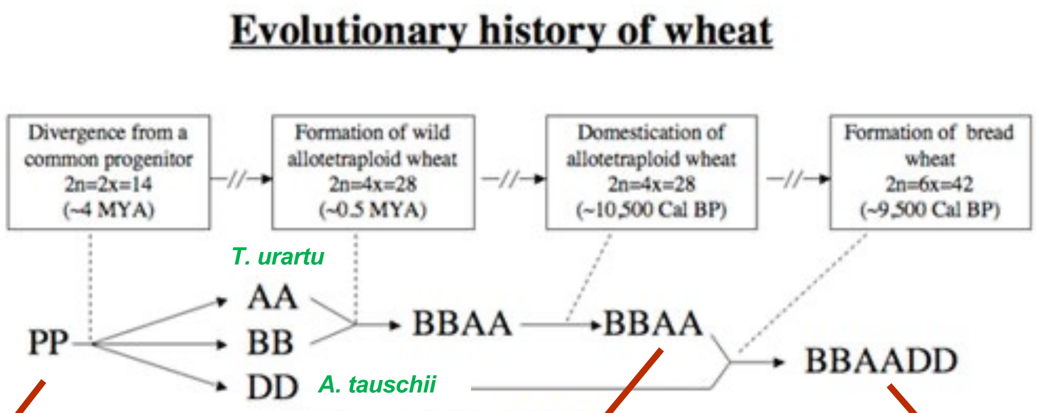
Complex hexaploid genome

Wheat and relative species - spikes and grains



Shewry 2009

Wheat evolution through polyploidization



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

Durum wheat
(2n=4x=28, tetraploid)

Bread wheat
(2n=6x=42, hexaploid)

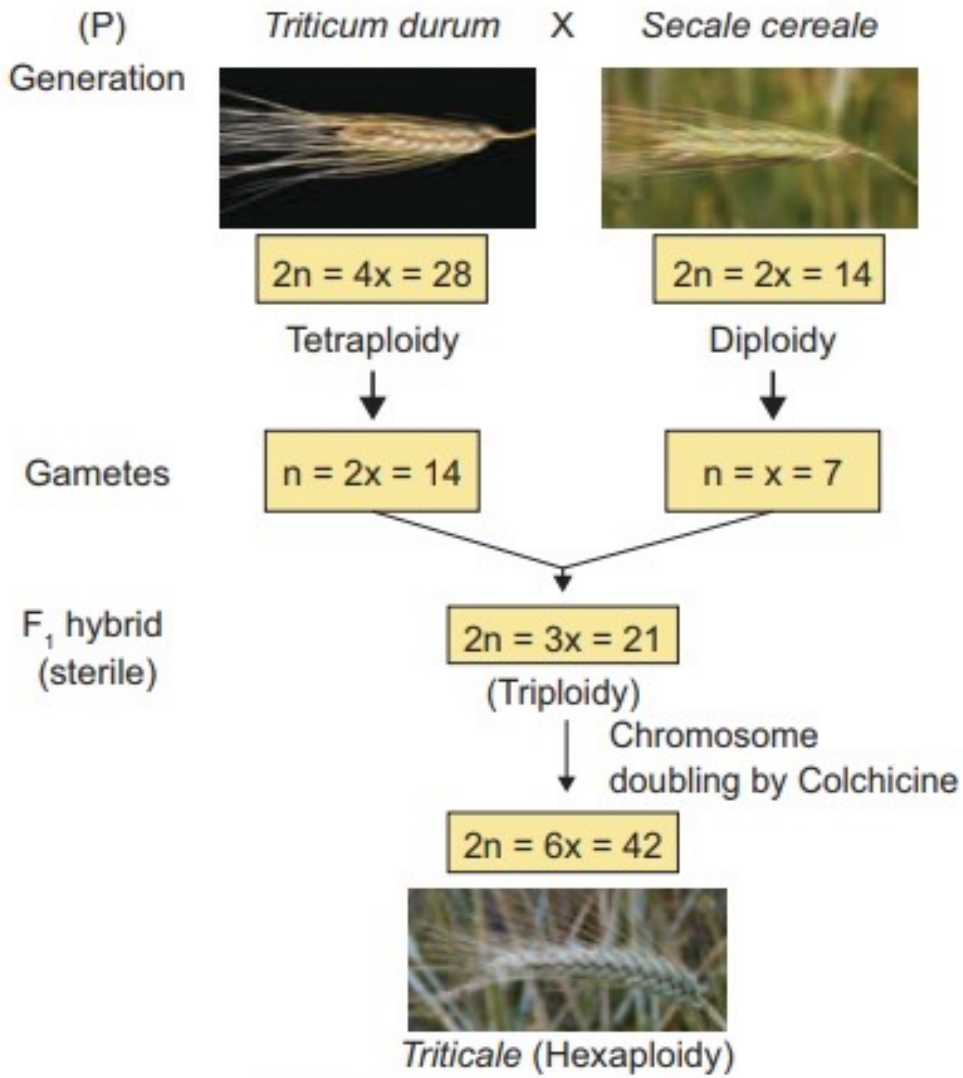
Levy 2007

Evolutionary History of Wheat

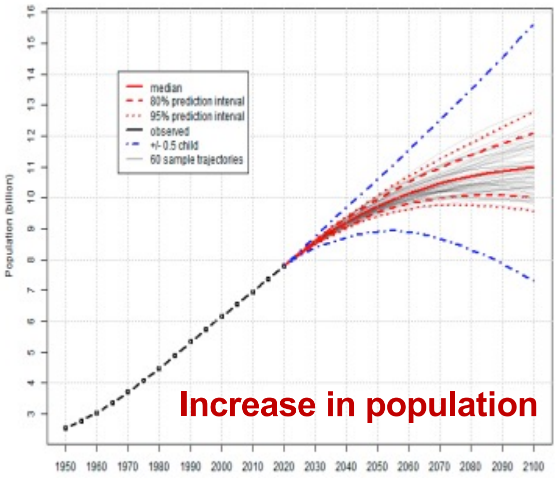
https://colostate.pressbooks.pub/cropwildrelatives/chapter/wheat-breeding-with-crop-wild-relatives/

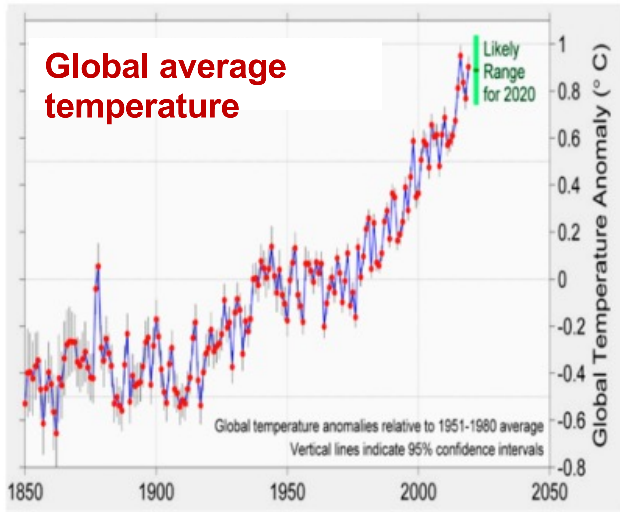
Synthetic allopolyploid: **triticale**

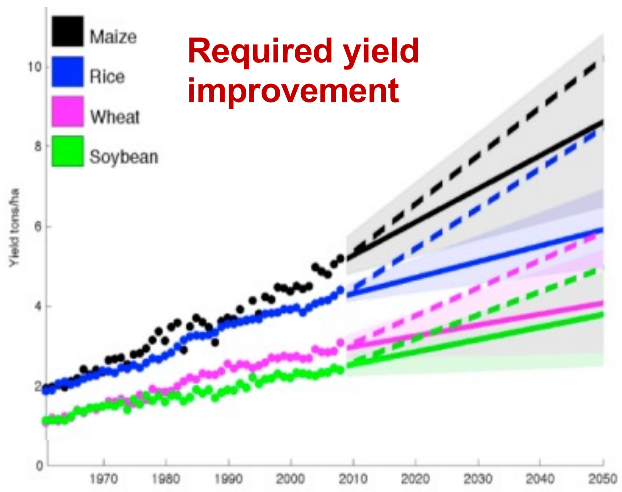
Seeds of wheat (left), triticale (centre) and rye (right)
(Source: A. Stephen Wilson 1875)



Future increase in food demand







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

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 environmental **stress conditions**

Particularly considering the **genetically eroded commercial varieties**

← decades of homogenization through breeding

Colecção de variedades tradicionais de trigo mole e trigo duro


Bolefim 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

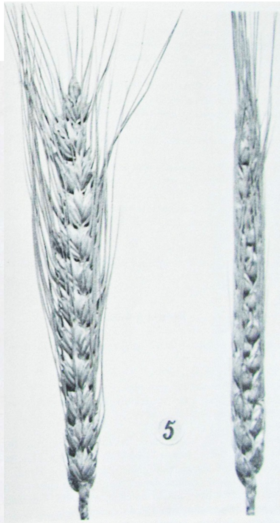
OU DE HÁ MUITO CULTIVADOS NO PAÍS

(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



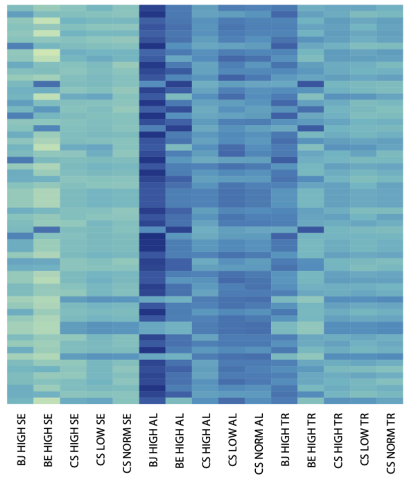
Vasconcelos, J. C. (1933). **Trigos portuguêses ou de há muito cultivados no país**. Subsídios para o seu estudo botânico. *Bol. Agric.* 1, 2, 1–150.

Heat effect on **wheat** grain

- level of peptides involved in **celiac disease**

Evaluation of expression level of 63 genes coding <u>peptides with known immunoreactivity</u>

high temperature → <u>increased</u> expression levels



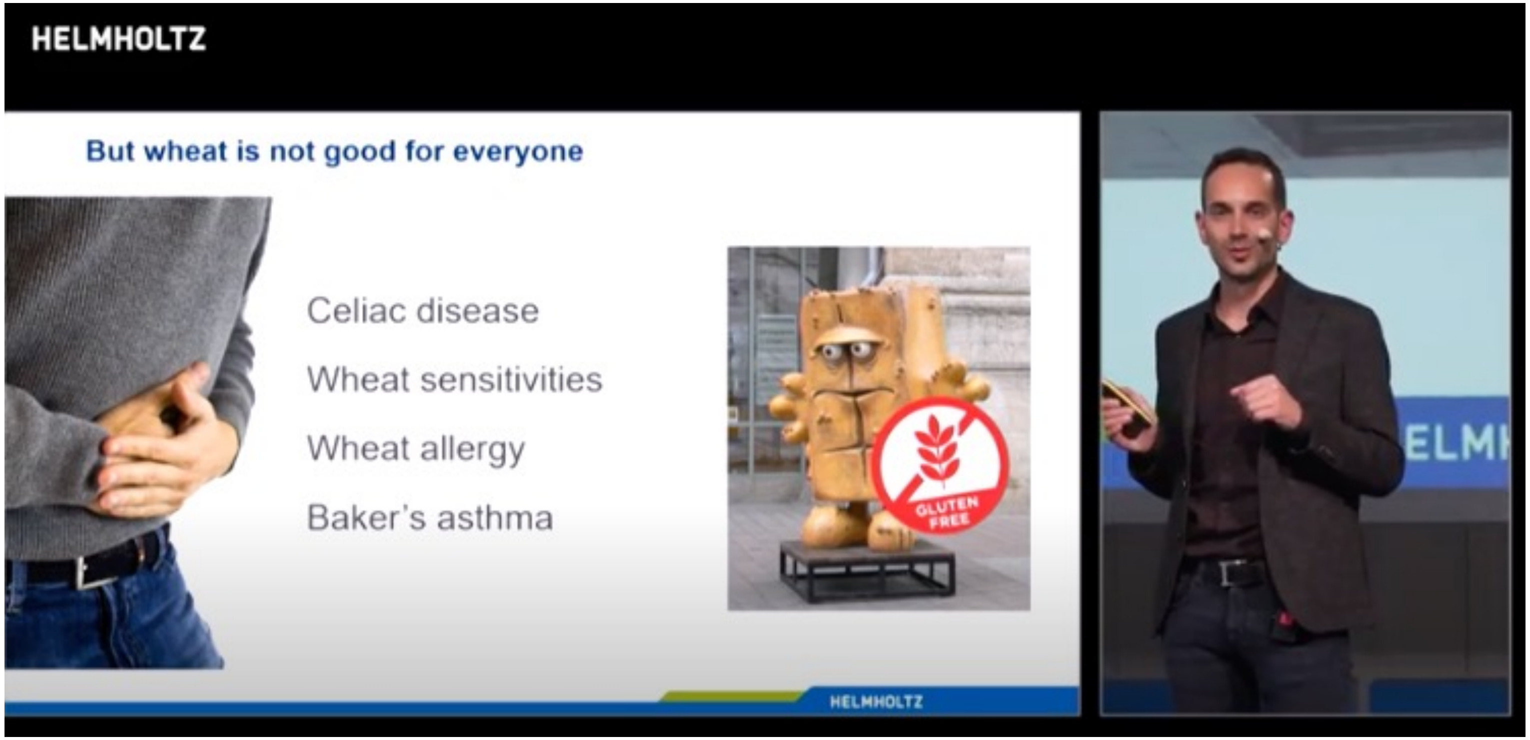
Climate change does impact wheat allergen expression

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



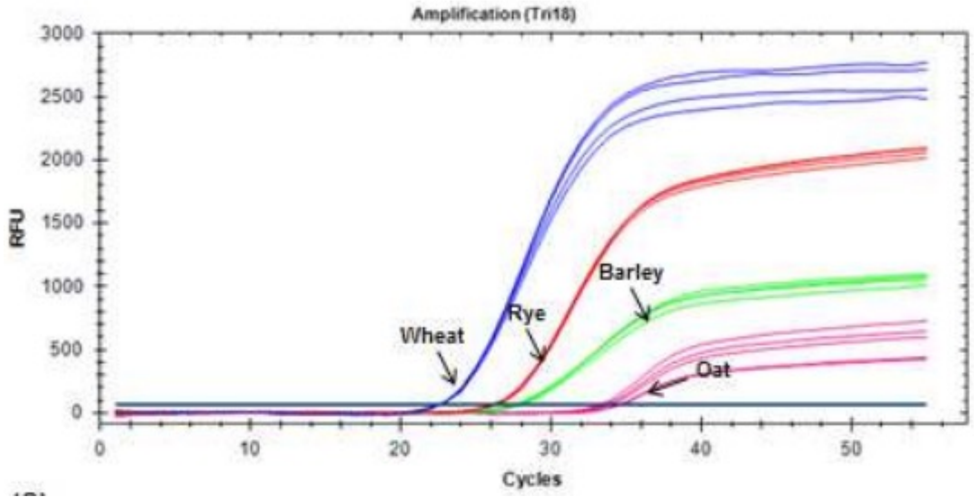
https://www.youtube.com/watch?v=6l1dU2-tdVU

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]

Detection of gluten in foods



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

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.

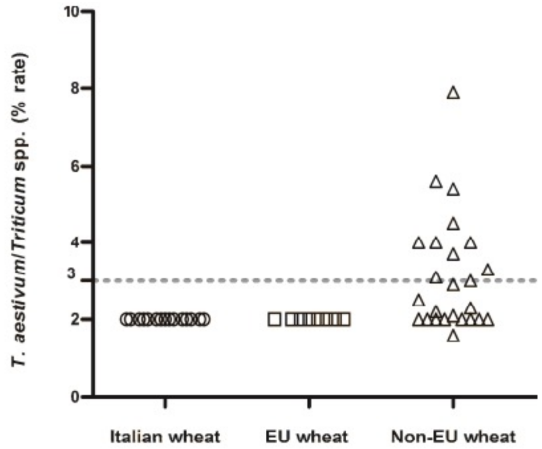
→ This PCR systems can be used as tools to confirm the presence of **gluten-containing cereals** in foods, towards the safety of celiac patients

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%**.

→ **protection of traditional pasta**

Molecular quantification method

- **DNA extraction** from semolina
- real-time **PCR** targeting **gluten** **genes** (gliadin and glutenin)

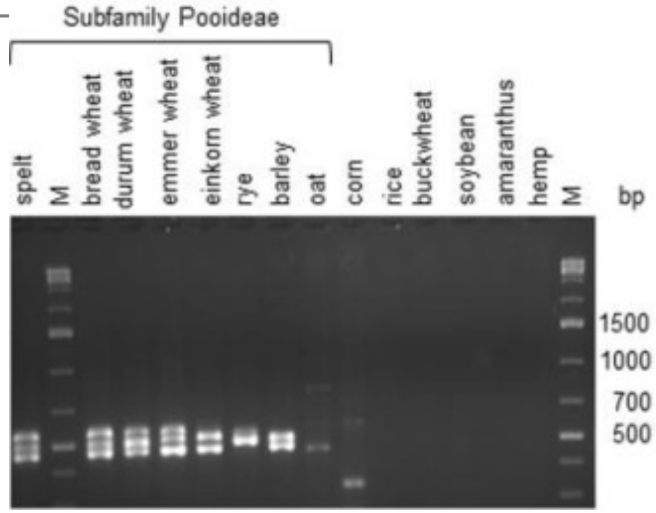
→ allow a specific and sensitive detection

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

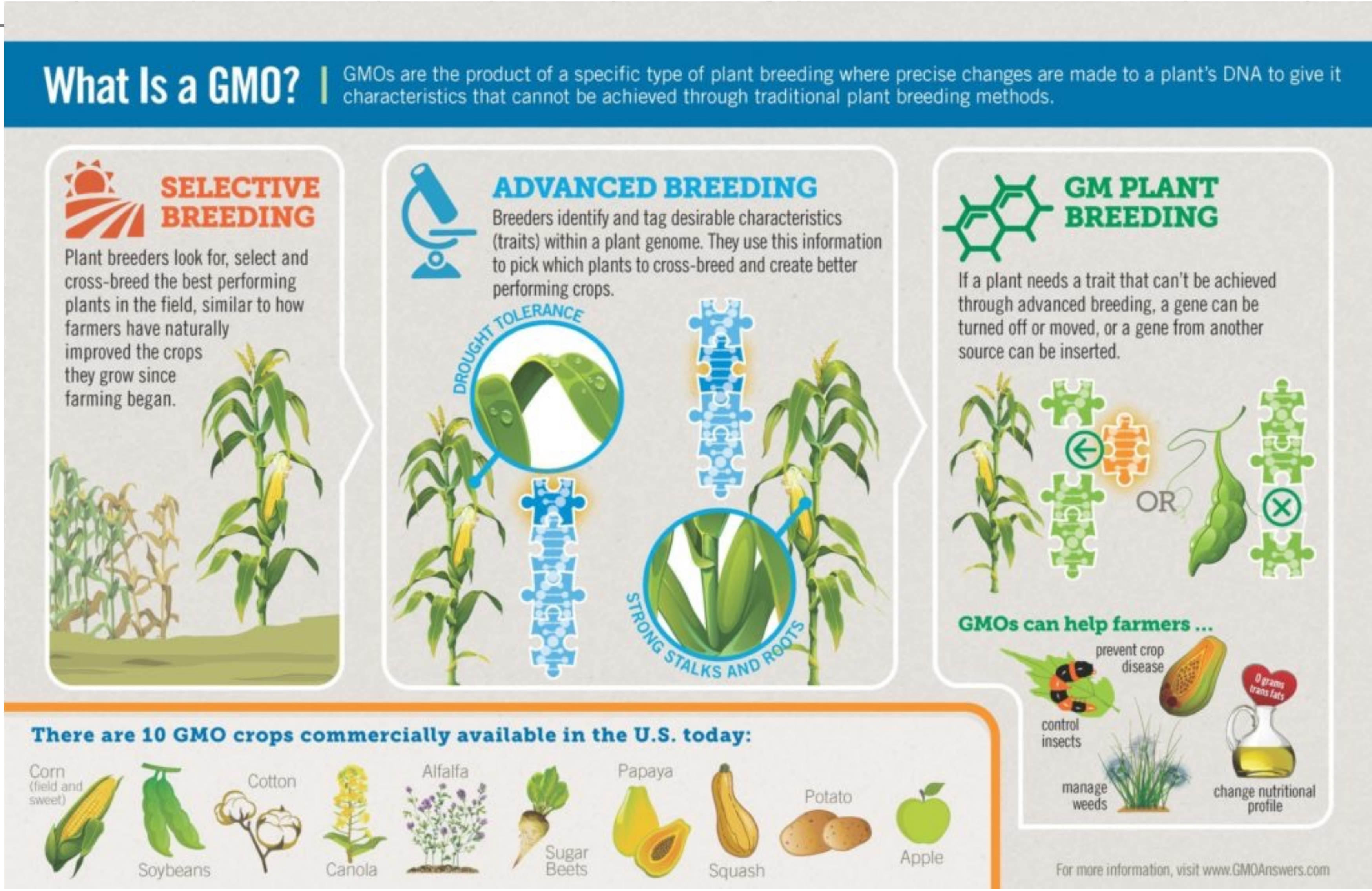
Pasta, bread and cookies, made with mixed flours containing **ancient wheat species** and **other cereals**

→ analytical methods to **food composition authenticity**

Tubulin-based polymorphism (TBP)

→ discriminate <u>different cereal species</u>

Sensitivity of 0.5–1% to 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

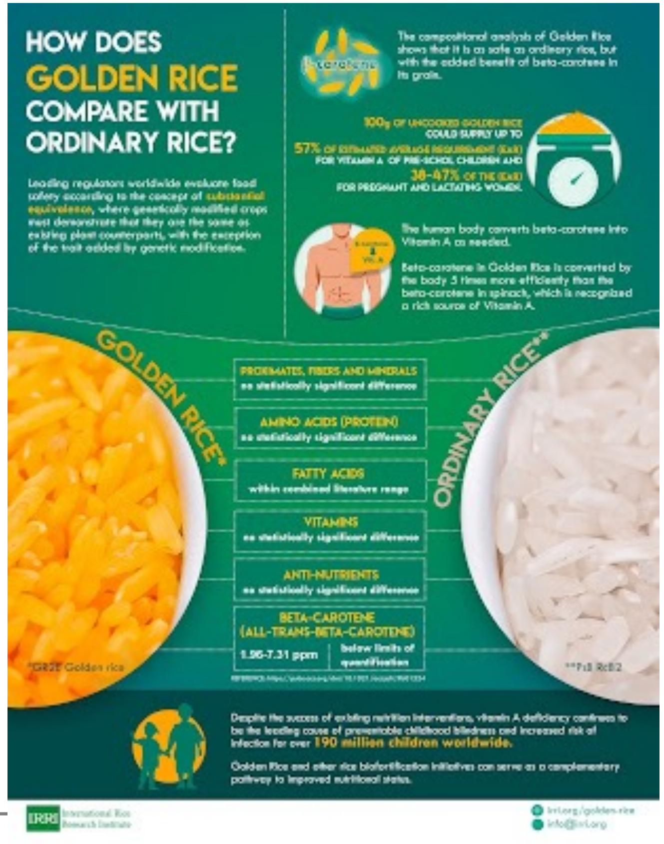
Estima-se que um milhão de crianças morre todos os anos com **deficiência em vitamina A** na Ásia onde a base da alimentação é o **arroz**.

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



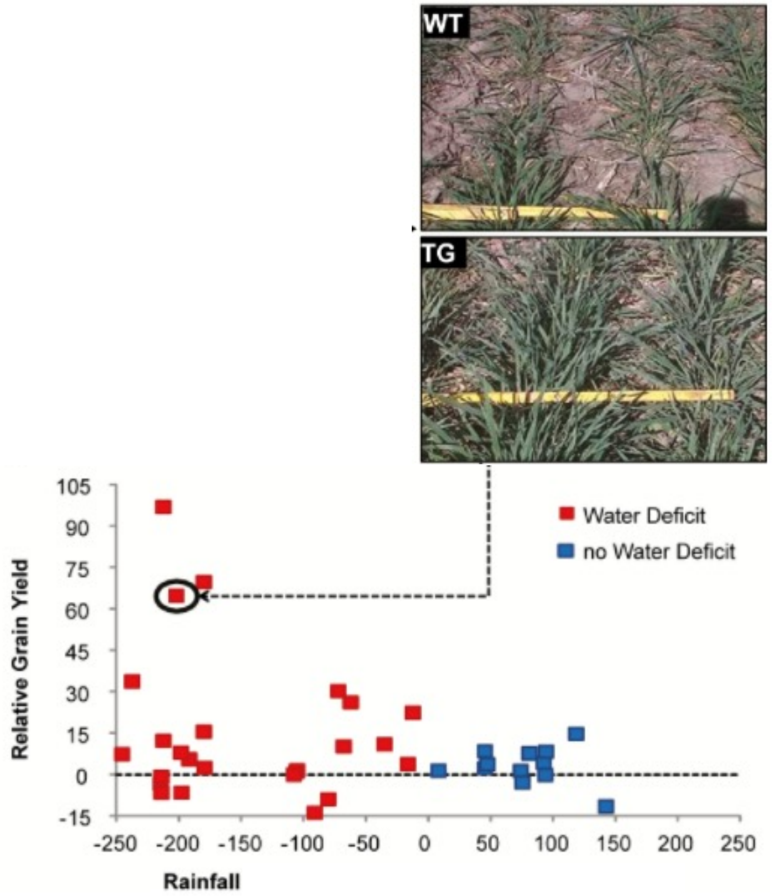
OGM Wheat IND-ØØ412-7
Drought tolerance

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 <u>equivalent to non-transgenic wheat</u>

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