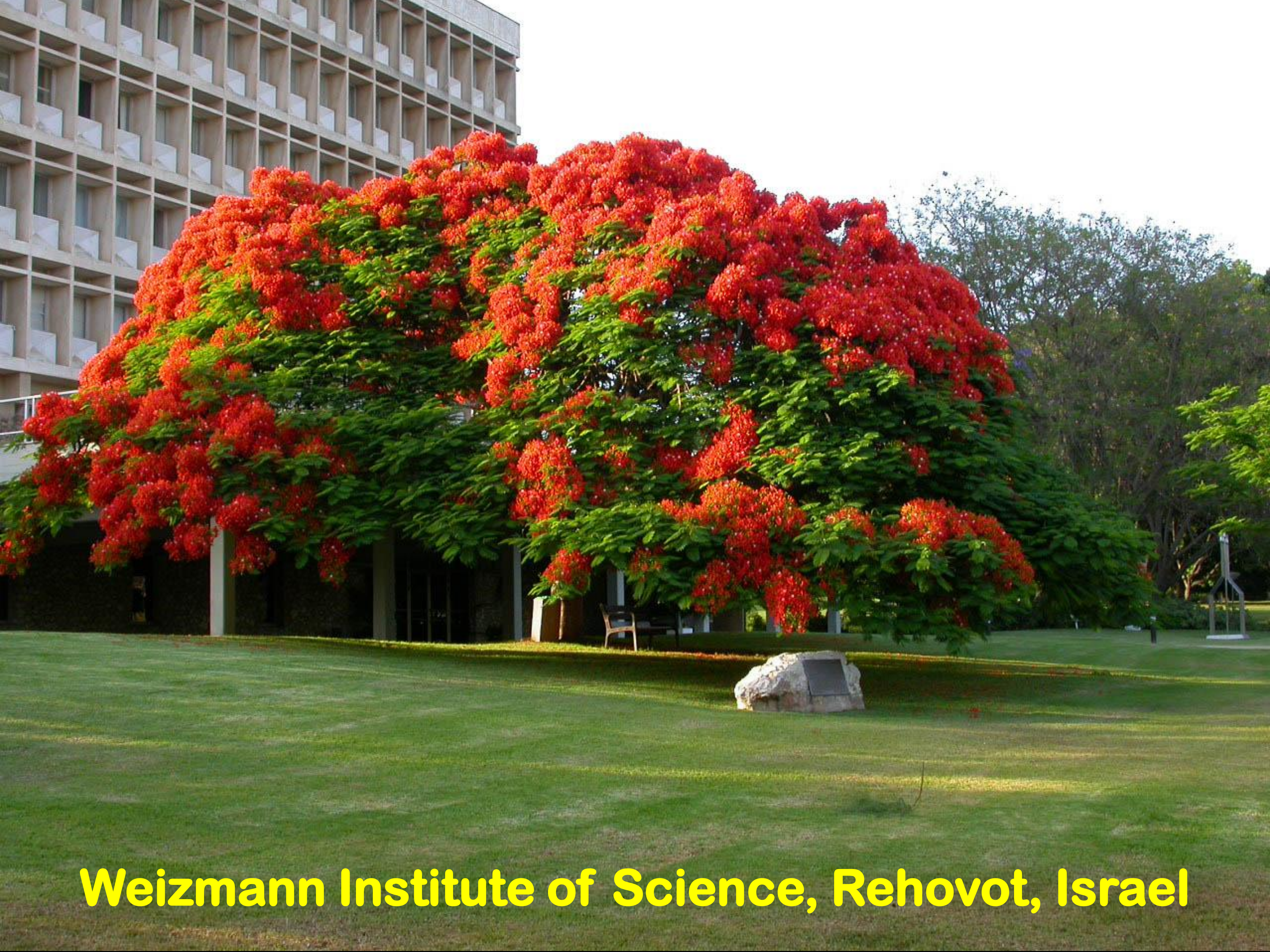
Wheat domestication: When, Where and How– insight from modern genetics

Avi Levy, Department of Plant Sciences, Weizmann Institute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Feinberg Graduate School	Faculty of Biology	Faculty of Biochemistry	Faculty of Chemistry	Faculty of Physics	Faculty of Mathematics & Computer Sciences
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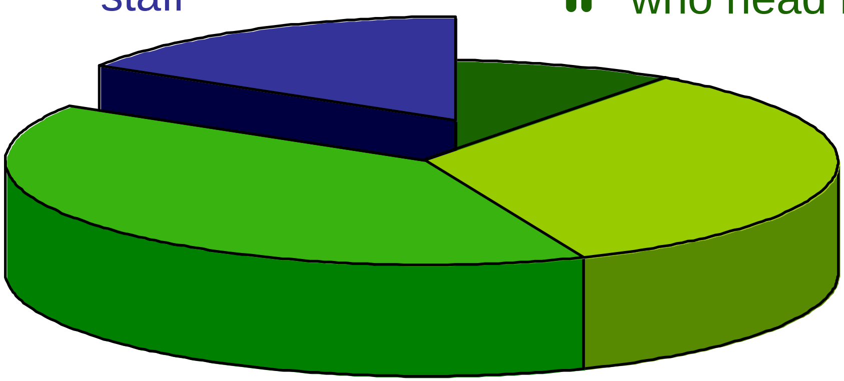
400 administrative staff

250 professors who head research groups



1,000 M.Sc. and Ph.D. students

850 scientists with Ph.D. degree, engineers and technicians

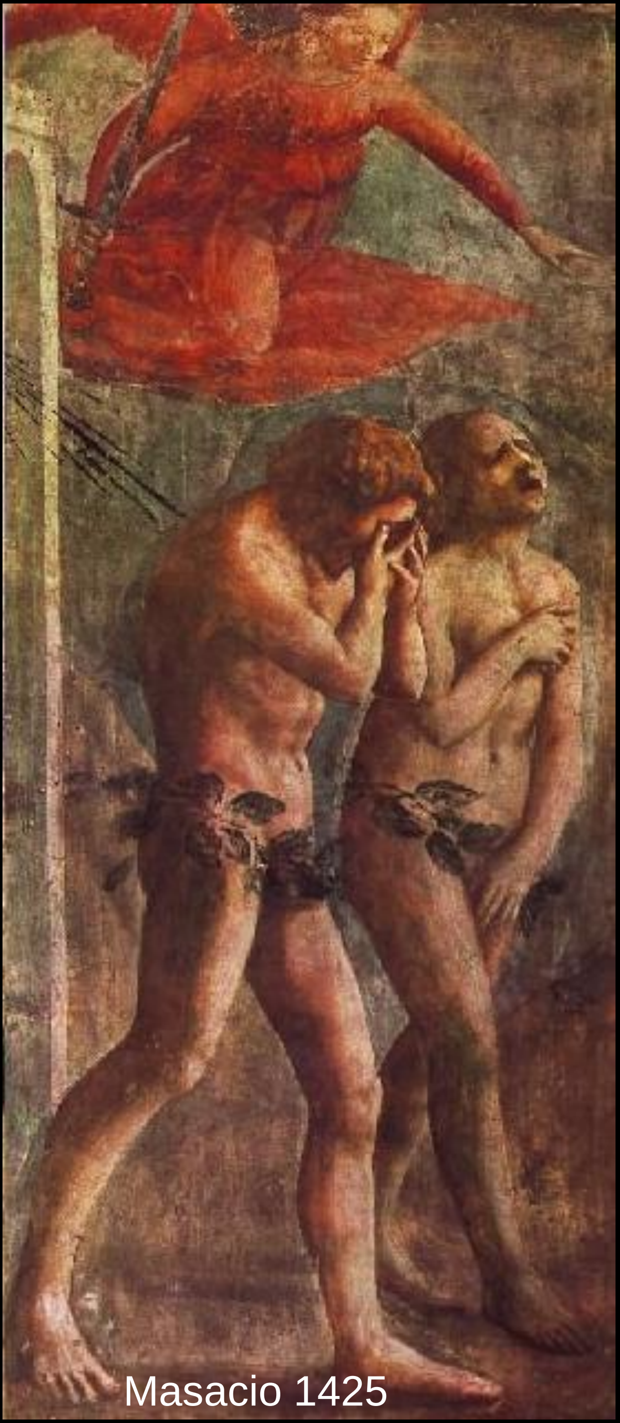
Triticum turgidum var dicoccoides

Where Cereals come from?
a gift from the gods?

Isis and Osiris

Demeter

Ceres



Or a curse?

In the sweat of thy face you shall eat bread….. (Genesis, The Bible)

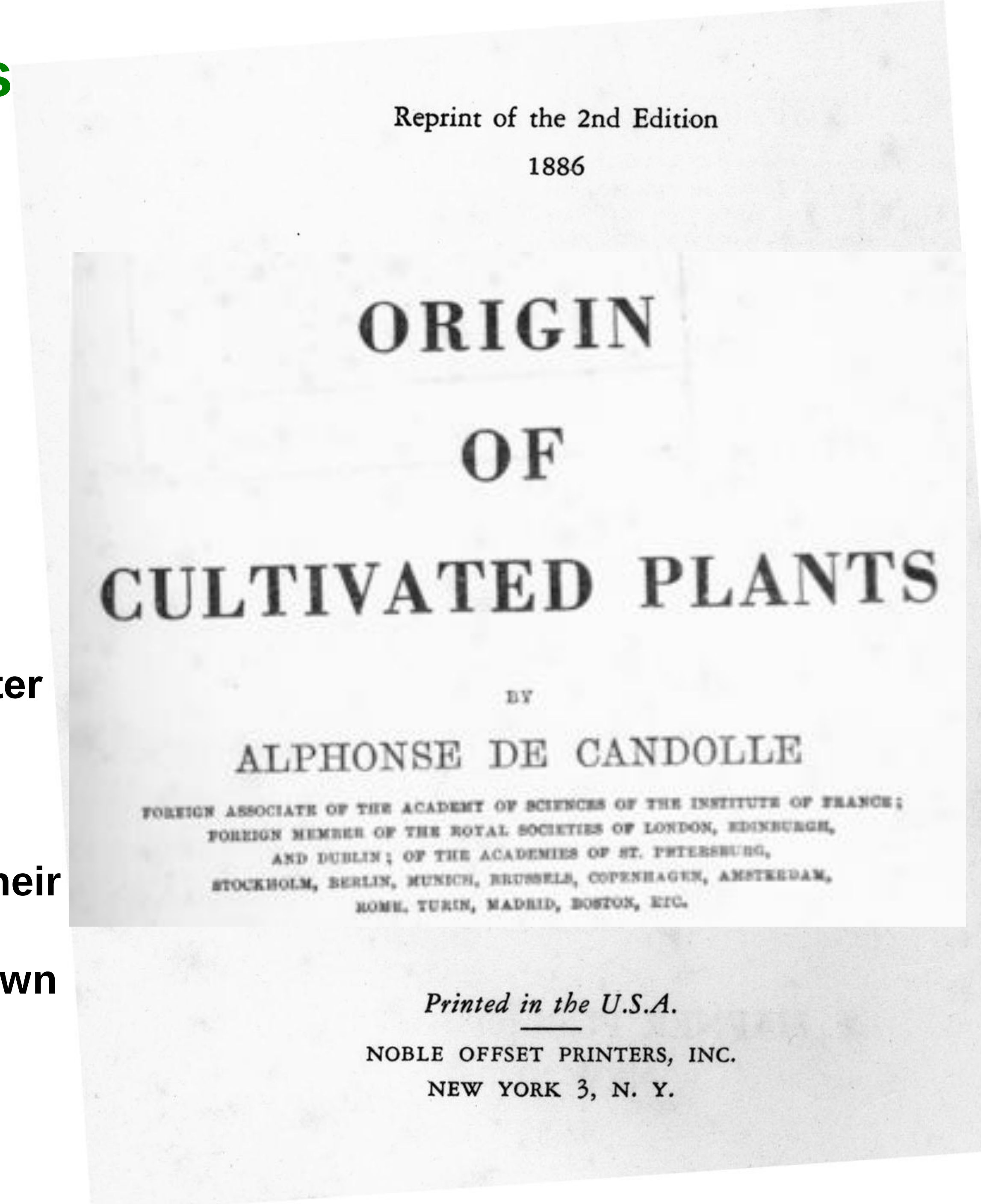
The origin of species by means of natural selection

By Charles Darwin, M.A., F.R.S.,

CHAPTER I.

VARIATION UNDER DOMESTICATION.

Causes of Variability …Character of Domestic Varieties-- Origin of Domestic Varieties from one or more Specie … Principles of Selection, anciently followed, their Effects …Methodical and Unconscious Selection .. Unknown Origin of our Domestic Productions …



Reprint of the 2nd Edition
1886

ORIGIN
OF
CULTIVATED PLANTS

BY

ALPHONSE DE CANDOLLE

FOREIGN ASSOCIATE OF THE ACADEMY OF SCIENCES OF THE INSTITUTE OF FRANCE;
FOREIGN MEMBER OF THE ROYAL SOCIETIES OF LONDON, EDINBURGH,
AND DUBLIN; OF THE ACADEMIES OF ST. PETERSBURG,
STOCKHOLM, BERLIN, MUNICH, BRUSSELS, COPENHAGEN, AMSTERDAM,
ROME, TURIN, MADRID, BOSTON, ETC.

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The study of the origin of domesticated plants is based on evidence from the following disciplines:

- Folkloristic
- Archaeology
- Botany
- Genetics and Genomics
- Chemistry
- Agronomy
- Climatology
- Anthropology
- History
- Linguistics

Tests used to identify the wild progenitor

- Classical taxonomic approach - morphological similarity.
- Reproductive barrier
- Cytogenetic analysis - chromosomal affinity
- Molecular biology - genetic distance based on markers, or comparative sequence analysis

The three cultivated species of wheat that were recognized by Linnaeus (Species Plantarum, 1753):

***Triticum monococcum* L.**

(*durum, pasta*)
***T. turgidum* L.**

(*bread*)
***T. aestivum* L.**

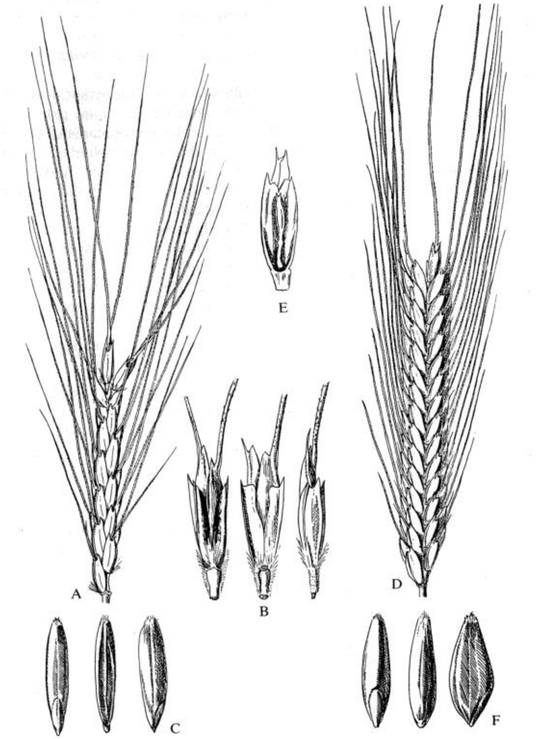
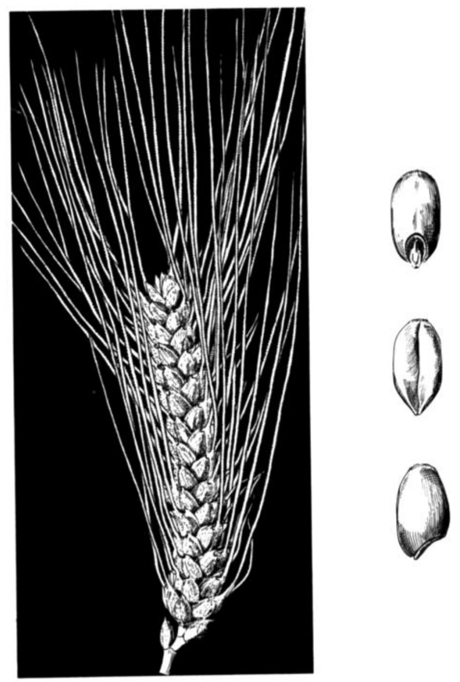
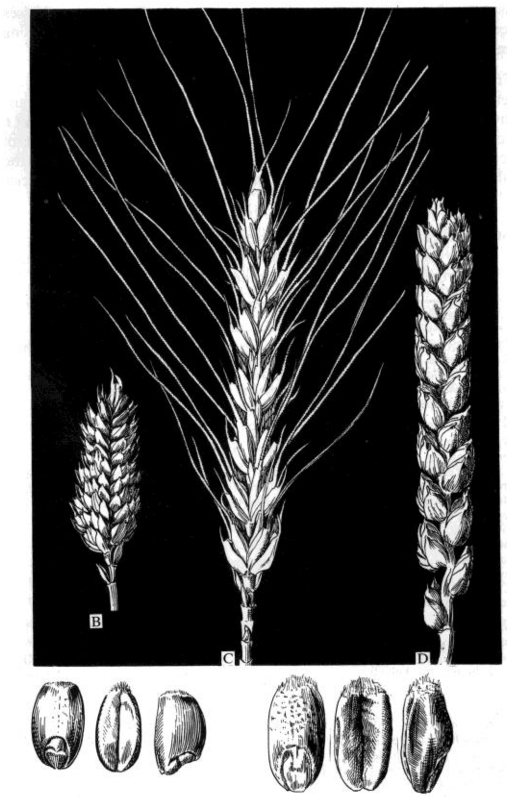


Fig. 2. Diploid einkorn wheats, *Triticum monococcum*. Left: A—ear (1:1), B—spikelet (2:1), and C—grain (3:1) of wild einkorn, *T. monococcum* subsp. *boeoticum*. Right: D—ear (1:1), E—spikelet (2:1), and F—grain (3:1) of cultivated einkorn, *T. monococcum* subsp. *monococcum*. (Schiemann 1948.)



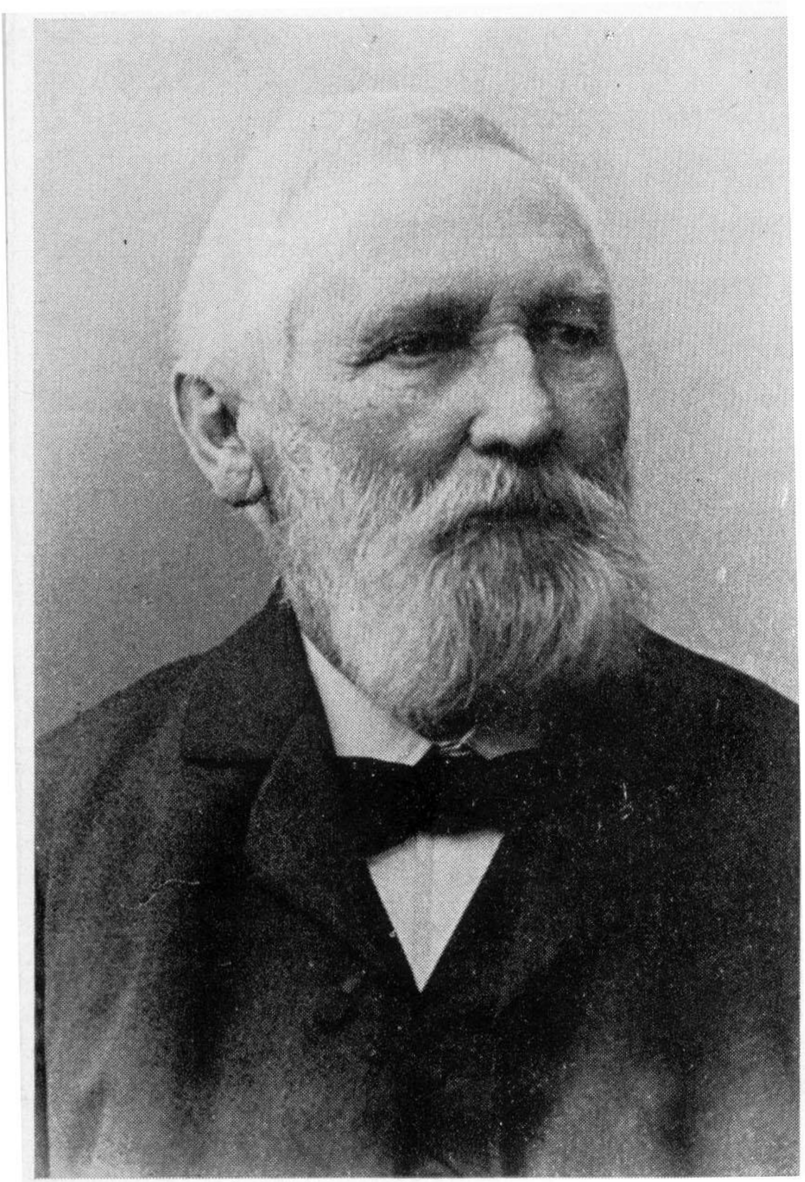


Theories concerning the site of wheat domestication at the end of the 19th century

Solms-Laubach ---> Central Asia (leading theory)

Much ---> South of the Baltic sea

De candolle ---> The Euphrates Basin



Friedrich körnicke
(1828 - 1908)

Found, among spikelets of wild barley collected by Kotschy in Rasheya (Syria) on the Northern slopes of mount Hermon, spikelets which were from a wild origin and looked like wild wheat – supporting De Candole's proposal of the Euphrates basin origin

The discovery of Wild emmer wheat in nature

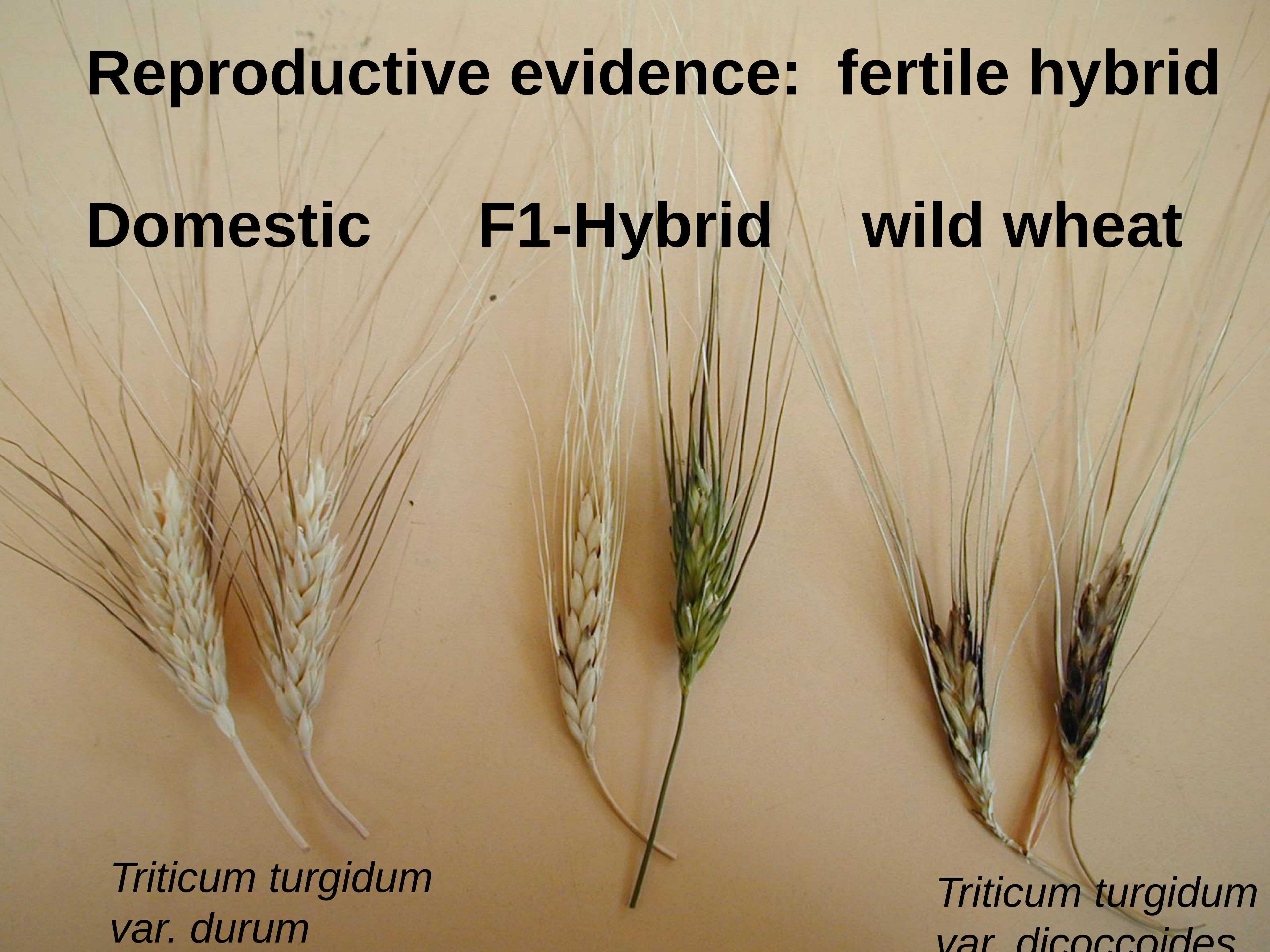
Aaron Aaronsohn
(1876-1919)

Wild wheat:
Triticum turgidum var dicoccoides

Domesticated wheat:
T. turgidum var duru

A fragile spike with a brittle rachis, 2 large grains per spikelet, strongly protected by stiff glumes

Reproductive evidence: fertile hybrid



The karyotypic evidence: the karyotype of domestic durum wheat is the same as that of wild *dicoccoides* wheat: n= 14 chromosomes

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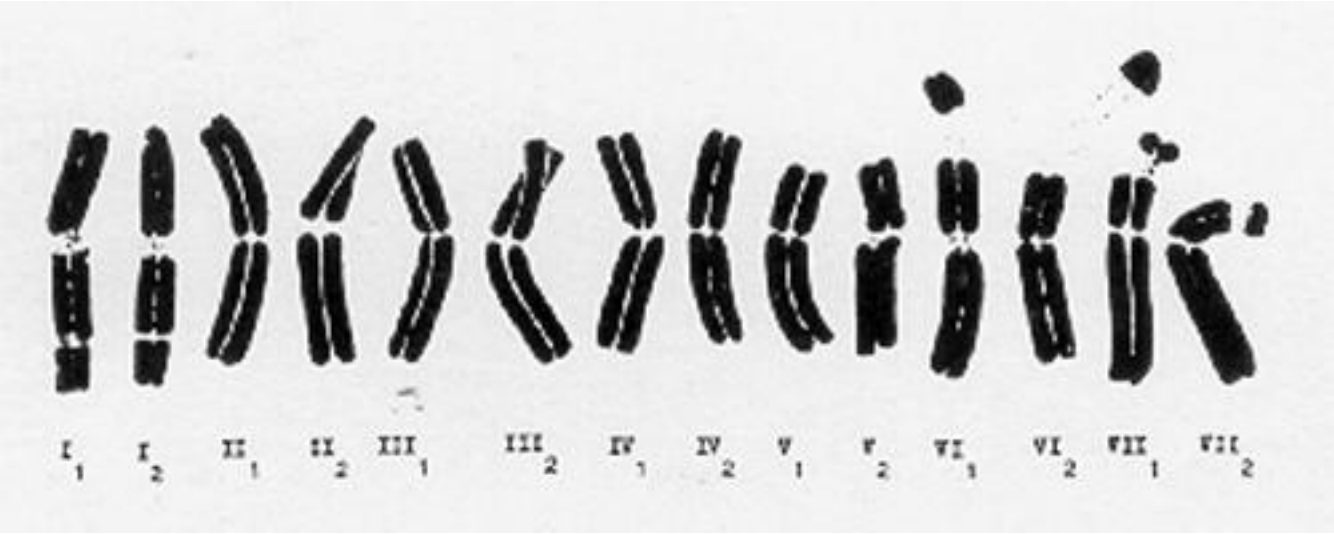
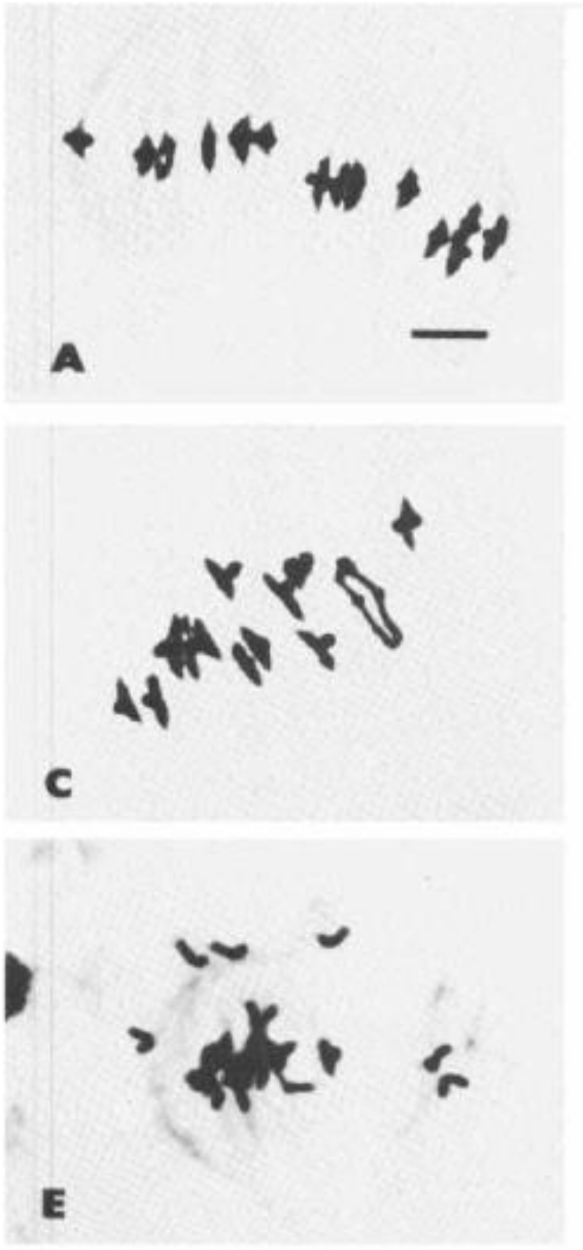
The karyotype of domestic Barley is the same as that of wild barley: n= 7 chromosomes





Full

Partial

Low ---> Different species

The cytogenetic evidence: Chromosome number is the same in durum and dicoccoides and there is full pairing between the chromosomes (2n=28), 14 pairs

Evolution of wheat: an history of hybridization, allopolyploidization and domestication

Triticum dicoccoides

T. dicoccum

T. durum

T. aestivum

Divergence from a common progenitor
2n=2x=14
(~4 MYA)

→

Formation of wild wheat
2n=4x=28
(~0.5 MYA)

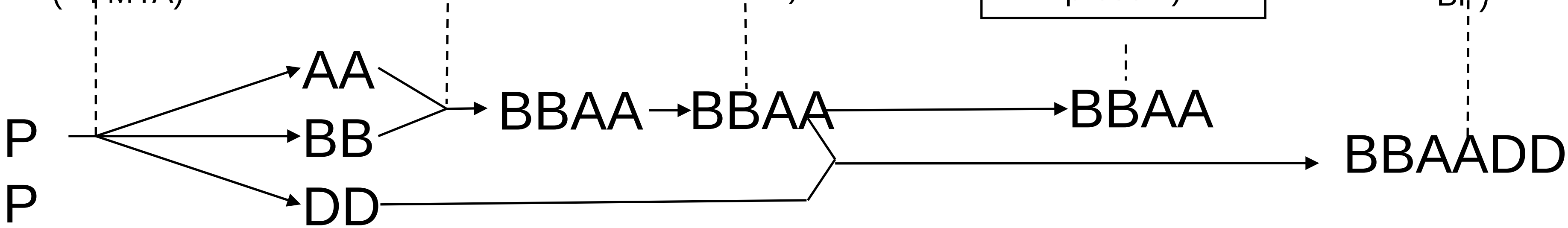
→

Domesticated primitive wheat
2n=4x=28
(~10,500 Cal BP)

→

Durum wheat (Landraces or modern varieties)
2n=4x=28
(few hundred YA-present)

Formation of bread wheat
2n=6x=42
(~9,500 Cal BP)



Classification of the species of *Triticum* (after van Slageren, 1994)

Species	Genomes	Wild	Domesticated
Diploid (2n=14)			
T. urartu	A	all	-
T. monococcum	A^m	ssp. *aegilopoides* (wild einkorn)	ssp. *monococcum* (domest. einkorn)
Tetraploid (2n=28)			
T. timopheevii	GA	ssp. *armeniacum*	ssp. *timopheevii*
T. turgidum	BA	ssp. *dicoccoides* (wild emmer)	ssp. *dicoccon* ssp. *parvicoccum** ssp. *durum* ssp. *turgidum* ssp. *polonicum* ssp. *carthlicum*
Hexaploid (2n=42)			
T. zhukovskyi	GAA^m	-	ssp. *zhukovskyi*
T. aestivum	BAD	-	ssp. *spelta* ssp. *macha* ssp. *vavilovii* ssp. *aestivum* ssp. *compactum* ssp. *sphaerococcum*

* Extinct, described by Kislev (1980).

DNA marker evidence -- Salamini lab.

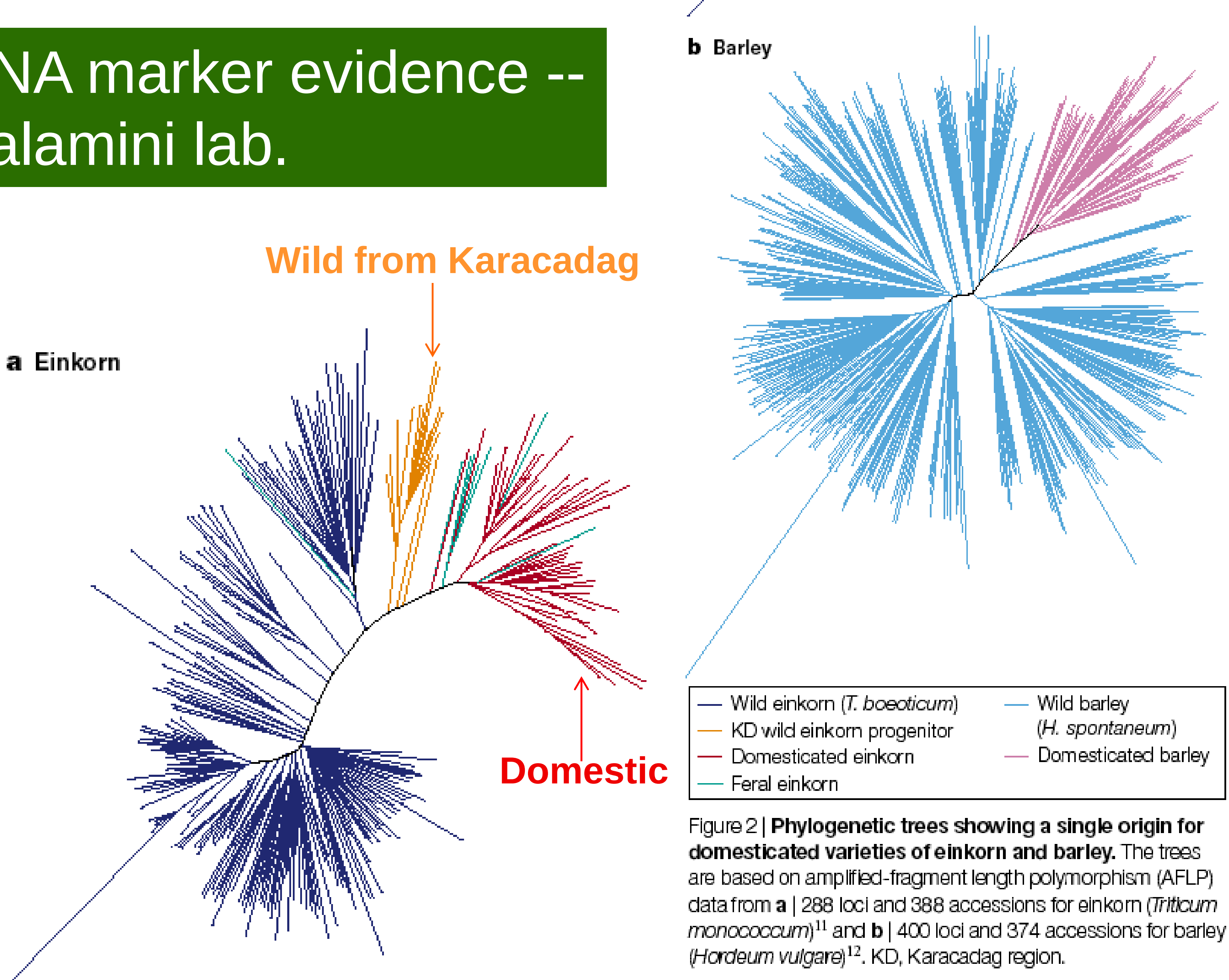
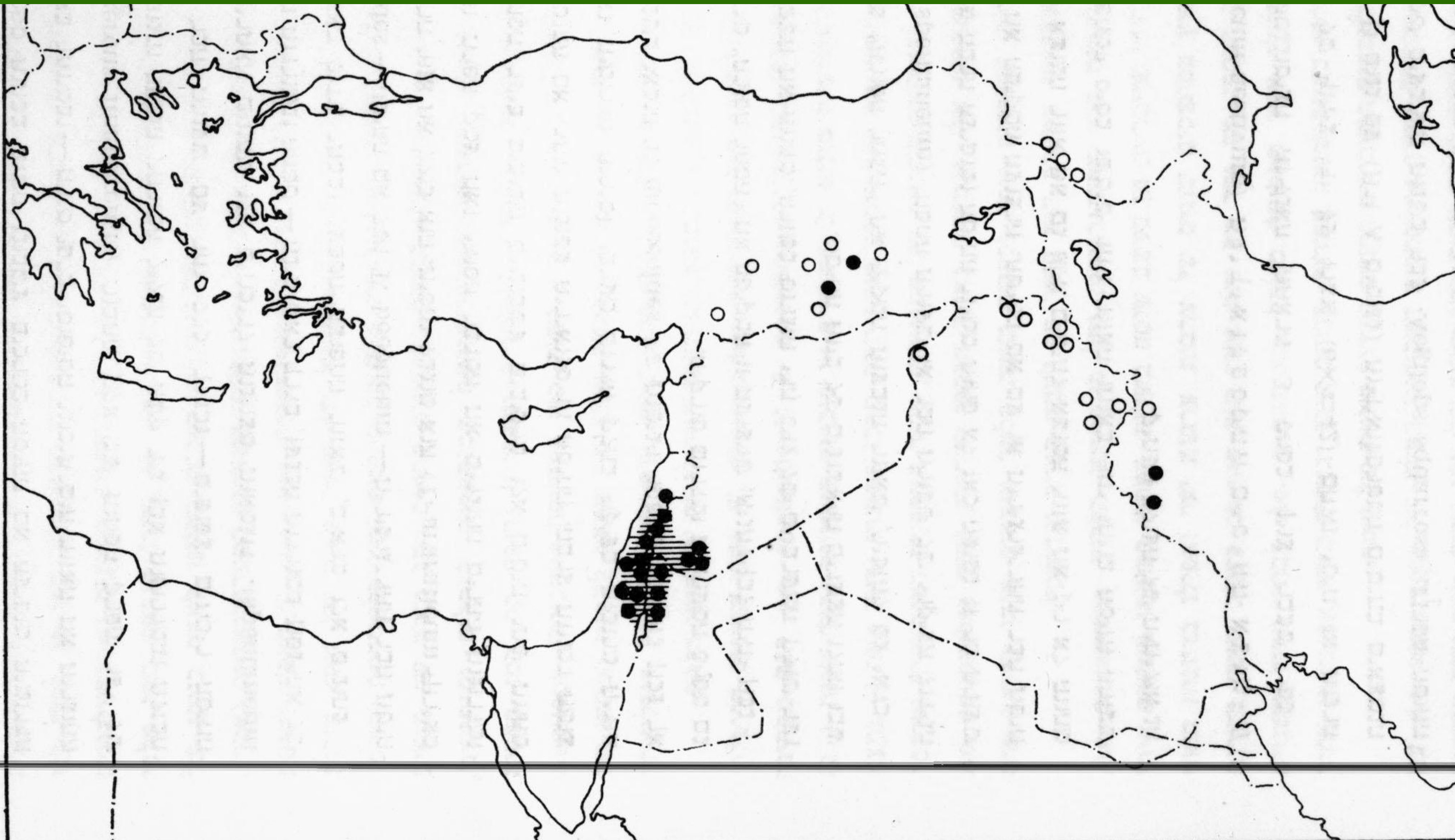


Figure 2 | **Phylogenetic trees showing a single origin for domesticated varieties of einkorn and barley.** The trees are based on amplified-fragment length polymorphism (AFLP) data from **a** | 288 loci and 388 accessions for einkorn (*Triticum monococcum*)[11] and **b** | 400 loci and 374 accessions for barley (*Hordeum vulgare*)[12]. KD, Karacadag region.

How did a species with a limited habitat become the largest grown crop worldwide – today 225 million hectares



מפה 2: תפוצת אם החיטה Triticum dicoccoides (עיגולים שחורים) ותפוצת החיטה הארמנית T. araraticum (עיגולים חלולים). האיזור העיקרי של אם החיטה מודגש בקווים.

Changes involved in the transition from wild into domesticated wheat

Wild form--------> Domesticated (hulled)------> Domesticated (free-threshi

Loss of seed dispersal mechanism

Loss of grain protectio n (stiff glumes)

Changes involved in the transition from wild into domesticated wheat

Selection for a more compact spike (Q)

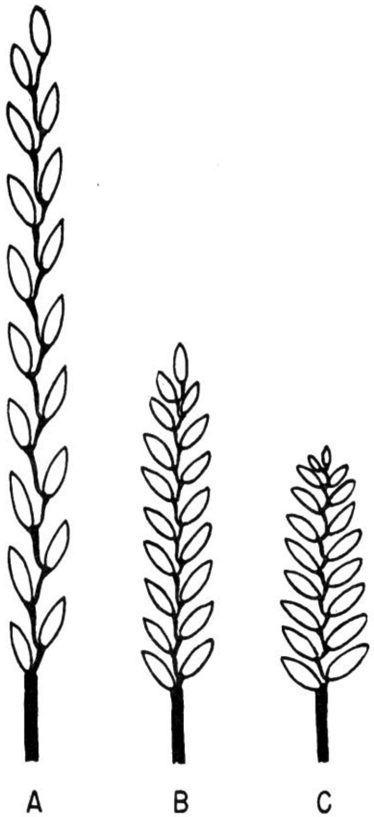


FIG. 1.—Diagrams of wheat heads of varying density. A, lax; B, dense; C, compact. (*After* Watkins (1930)).

Selection for multiple seeds per spikelets

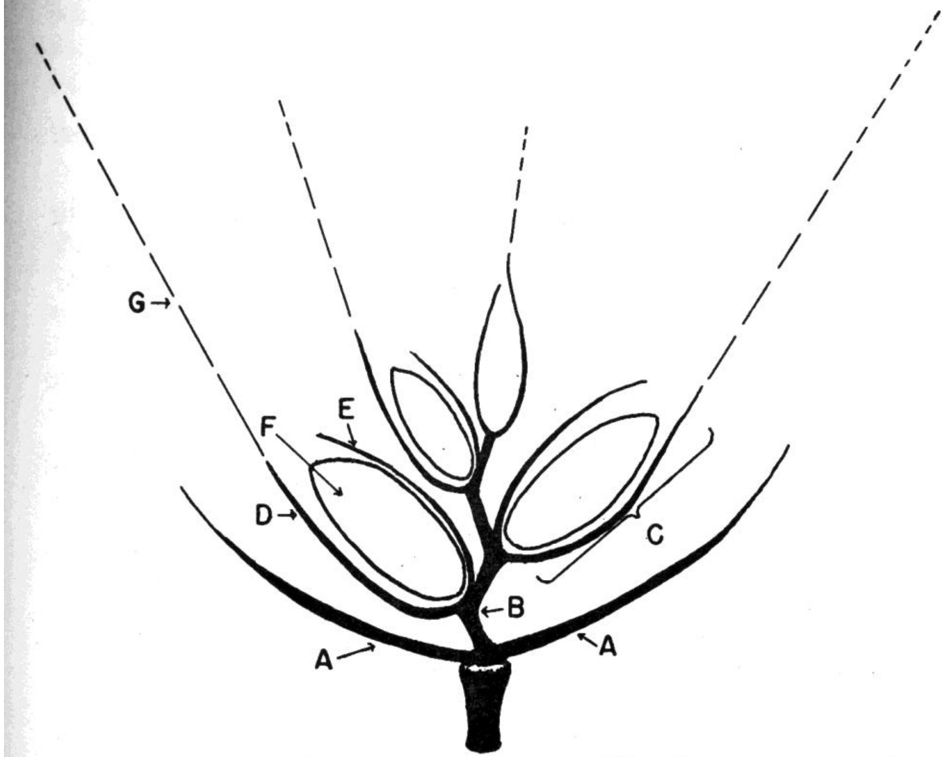


FIG. 2.—Diagram of a wheat spikelet. A, A, glumes; B, rachilla; C, floret; D, lemma; E, palea; F, grain; G, awn.

Evolution of tetraploid *turgidum* wheat, genome 2n=4x

T. durum

T. dicoccoides
(wild lines)

Fragile, hulled
2 grains/spikelet

→

T. dicoccum
(primitive varieties)

Non-Fragile, hulled
2 grains/spikelet

→

landraces

Non-Fragile,
Free threshing
> 2 grains/spikelet

→

Modern varieties

Non-Fragile,
Semi-dwarf
Free threshing
> 2
grains/spikelet

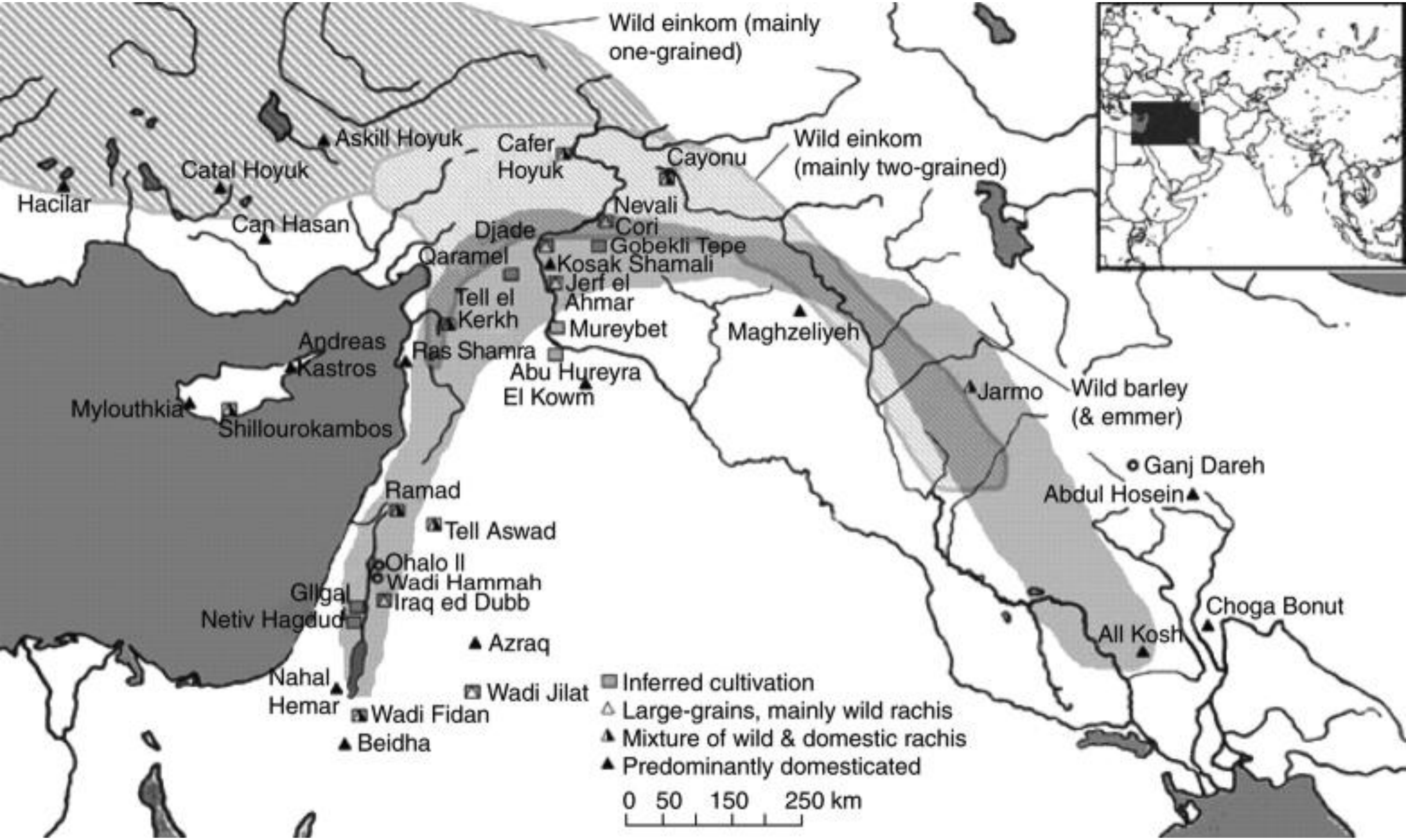
How did a species with a limited habitat become the largest grown crop worldwide – today 225 million hectares

The archaeological evidence:

Tracking the where and when of domestication through the analysis of botanical findings and diagnostic features such as:

- **Non fragile rachis,**
- **non-hulled types,**
- **ancient DNA**

Wild wheat together with early domestic types are found in Neolithic sites in the fertile crescent



Fuller et al. Annals of Botany 2007

Spikelets from fragile versus non-brittle spikes

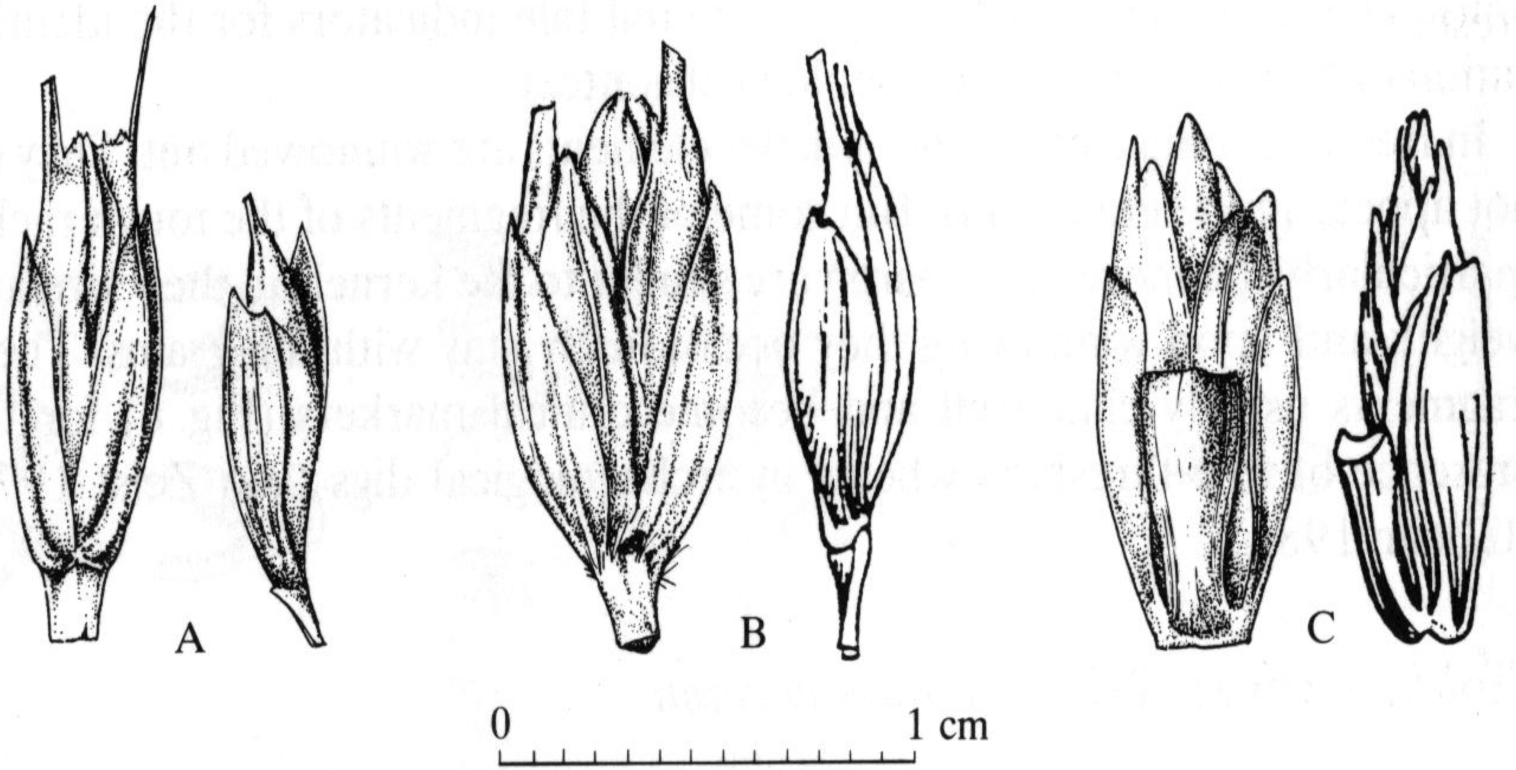
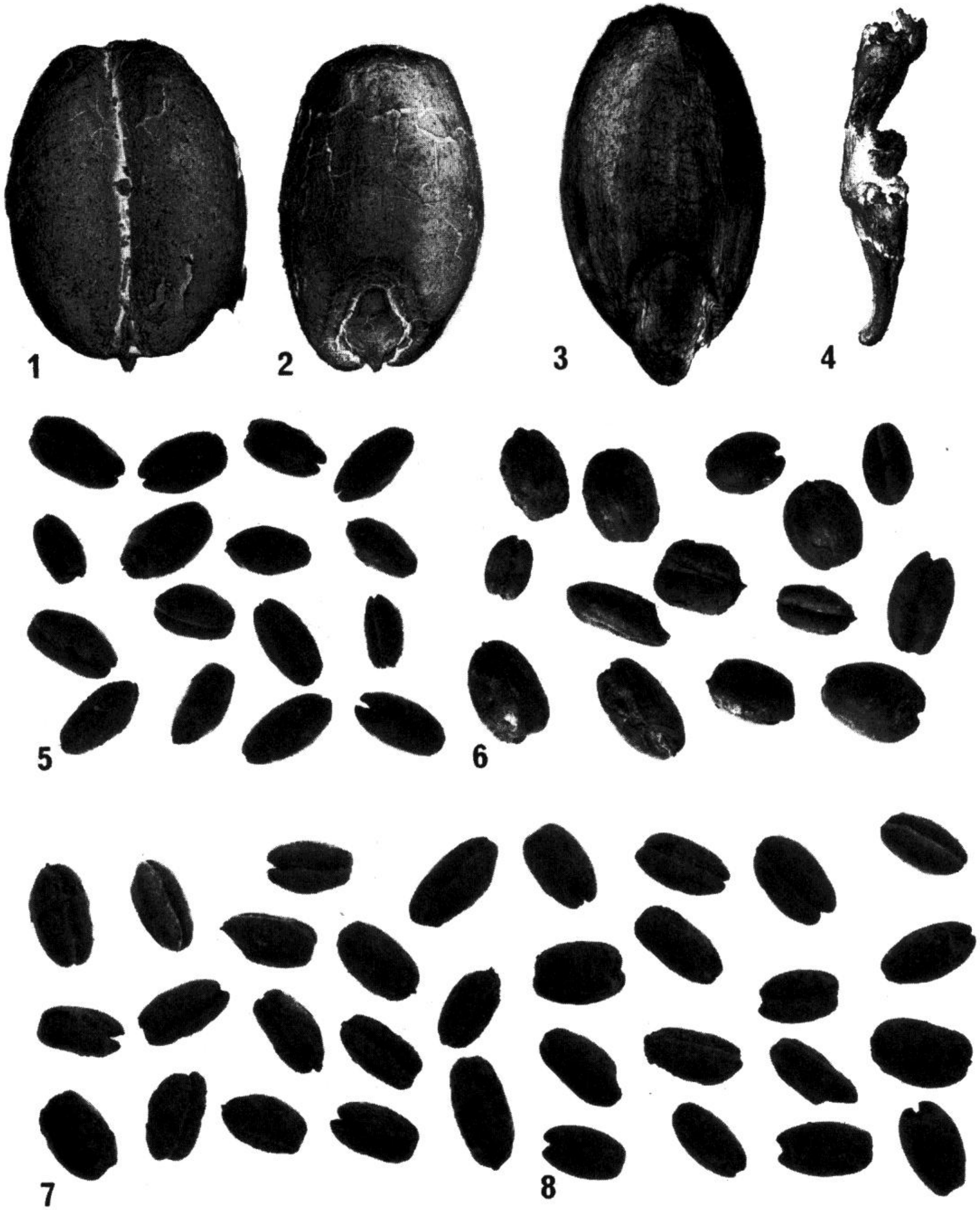


Fig. 6. The threshing products of the three main types of cultivated hulled wheats: A—Einkorn, *Triticum monococcum* subsp. *monococcum*. B—Emmer, *T. turgidum* subsp. *dicoccum*. C—Spelta, *T. aestivum* subsp. *spelta*. (Modern material.)

Glume forklets in archaeological remains

Fig. 7. Glume forklets, the diagnostic elements for the recognition of hulled wheats in archaeological remains. A—Einkorn. B—Emmer. Late Neolithic Goljamo Delcevo, Bulgaria. (Hopf 1975b.)



Rounded grains and rachis segments are diagnostic of domestic wheat types

Rachis segments

Modern wheat

Archaeological

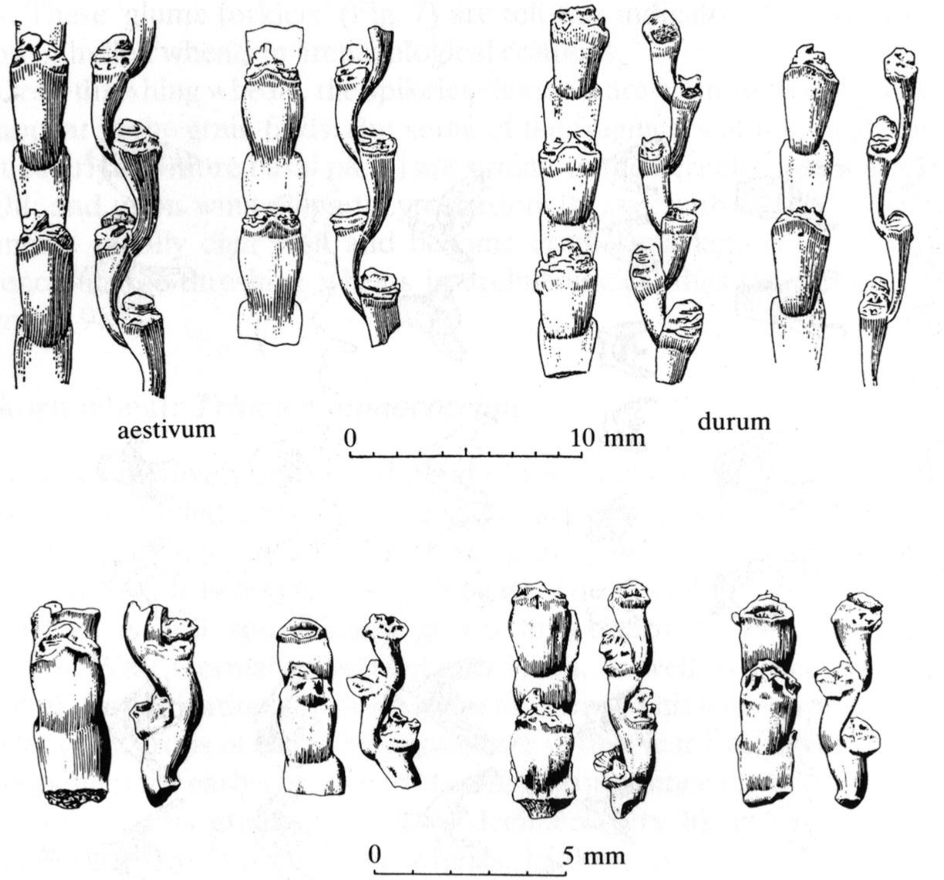


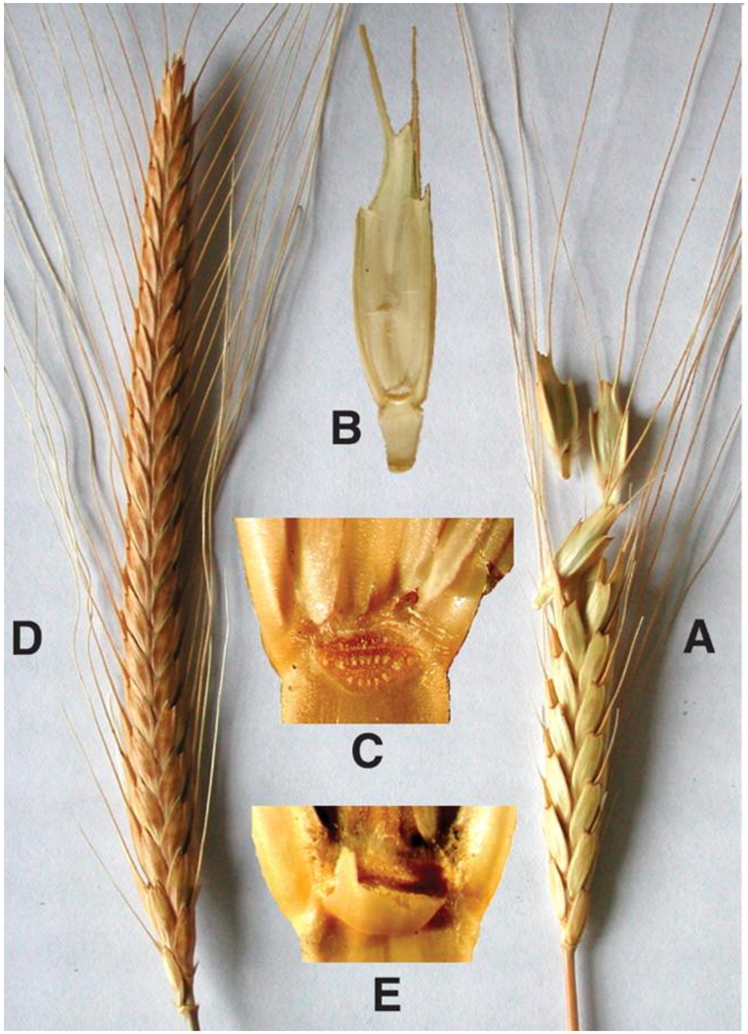
Fig. 8. Rachis segments, the diagnostic elements for the recognition of free-threshing wheats in archaeological remains. Upper row: rachis fragments separated from among threshed grains of modern bread wheat (left) and durum wheat (right). Lower row: Carbonized rachis fragments of free-threshing wheats. Neolithic Tell Ramad, Syria. (van Zeist 1976.)

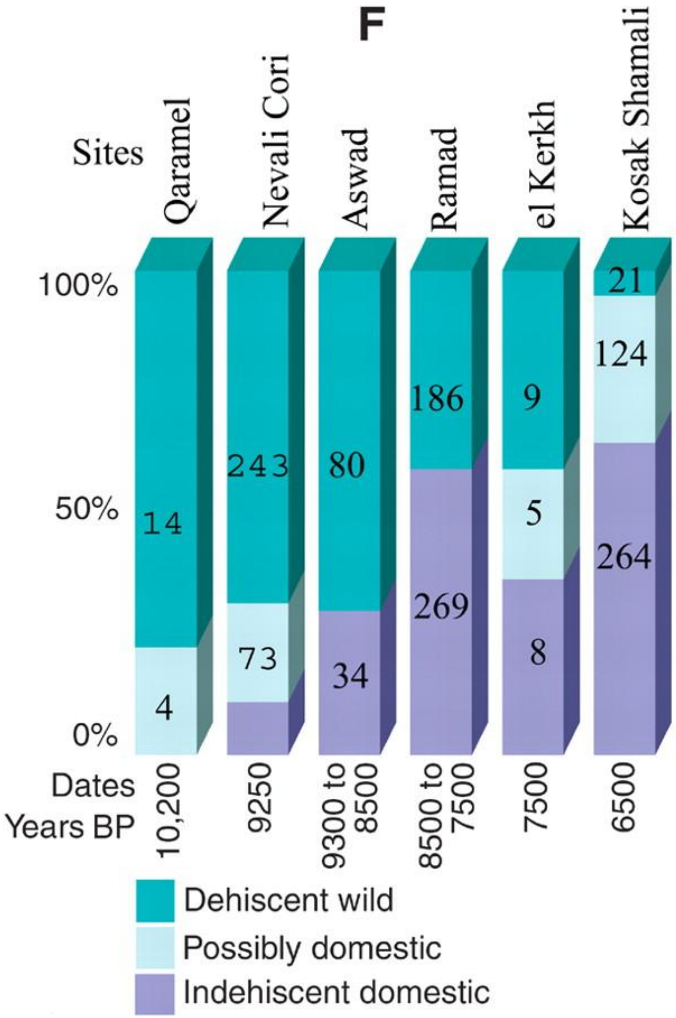
Barley spikelet fragments from Netiv Hagdud. The specimen on the left, characteristic of wild barley, shows a smooth, elliptical abscission scar at the connecting node, where the next spikelet up the ear has cleanly broken away. The "domesticated node" specimen on the right, in contrast, shows a small fragment of the lower part of the next spikelet up the ear still attached at the connecting node.

Barley spikelets from Netiv hagdud (early neolithic site), characteristic of wild (left= smooth abscission scar) or domesticated (right=roughly broken spikelet segment)

Modern examples of dehiscent wild (A, B, C) and domestic non dehiscent einkorn wheat (D, E)

Archaeological specimens of wheat and Barley sorted as wild/domestic/intermediate in sites from early and late Neolithic





K Tanno, and G Willcox Science 2006;311:1886-1886

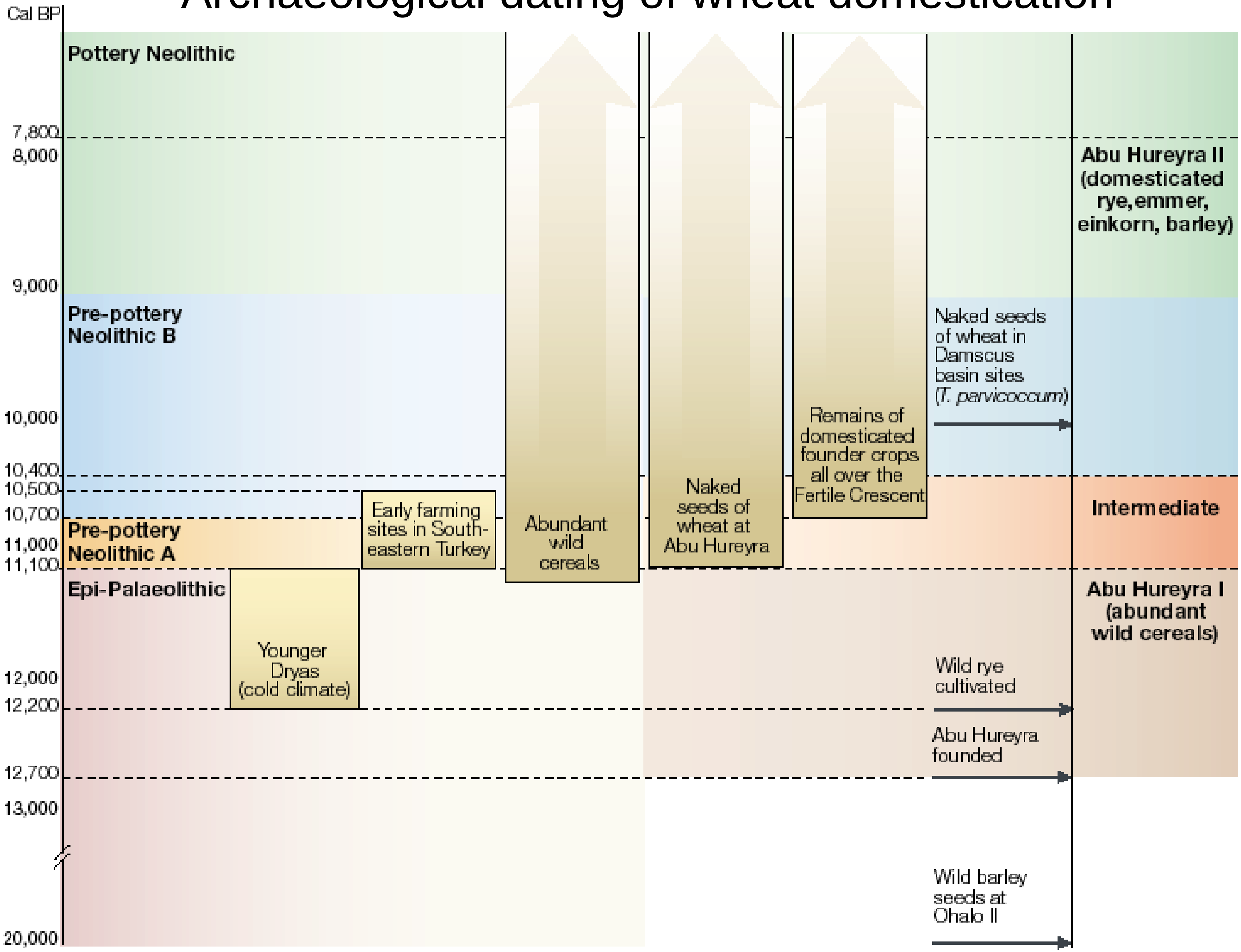
Published by AAAS

Chronology for the late Epipalaeolithic and the Neolithic periods in the Levant, the western flank of the Fertile Crescent

Date (BP)	Period	Major events in wheat cultivation
15,000 - 11,500	Late Epipalaeolithic (Natufian)	Harvesting from wild emmer and einkorn stands - agrotechnical development
11,500 - 10,500	Prepottery Neolithic A (PPNA)	Cultivation of brittle forms of emmer and einkorn - the first phase of cultivation
10,500 - 8,500	Prepottery Neolithic B (PPNB)	Appearance of non-brittle emmer and einkorn, naked tetraploid wheat, and naked hexaploid wheat - the second phase of cultivation
8,500 - 6,700	Pottery Neolithic	Spread of wheat culture to central Asia, southern Europe and Egypt - expansion of agriculture

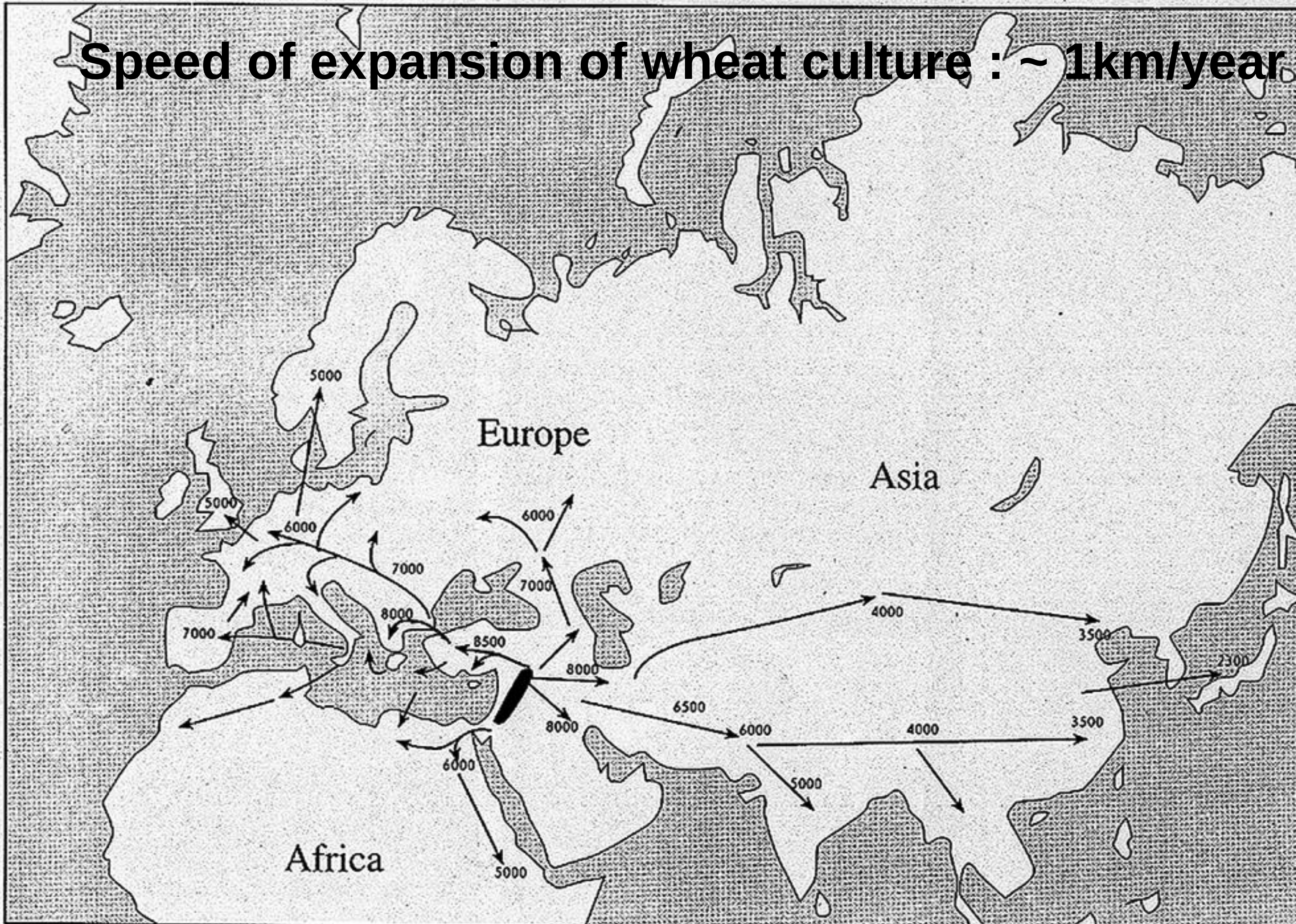
Moshe Feldman (Origin of cultivated wheats, 2001)

Archaeological dating of wheat domestication



Salamini lab. --Nature review

Speed of expansion of wheat culture : ~ 1km/year

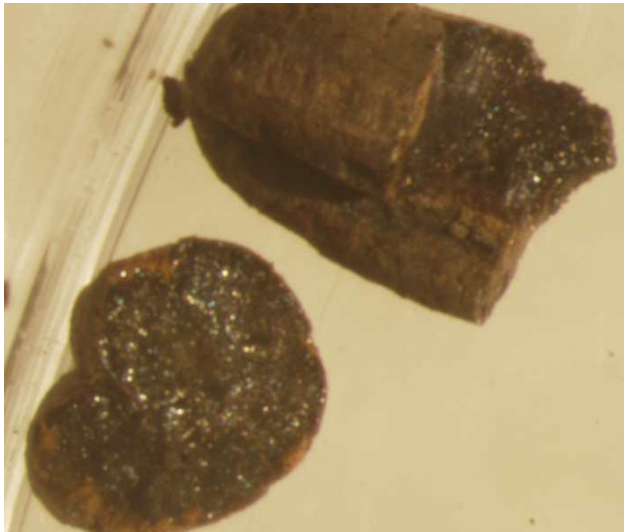


Map 4. Routes of expansion of wheat culture. Date of the earliest wheat cultivation in different sites is indicated b

Genetics provides unbiased but indirect evidence of location of domestication (could be affected by gene flow and genetic drift), can also provide some dating.

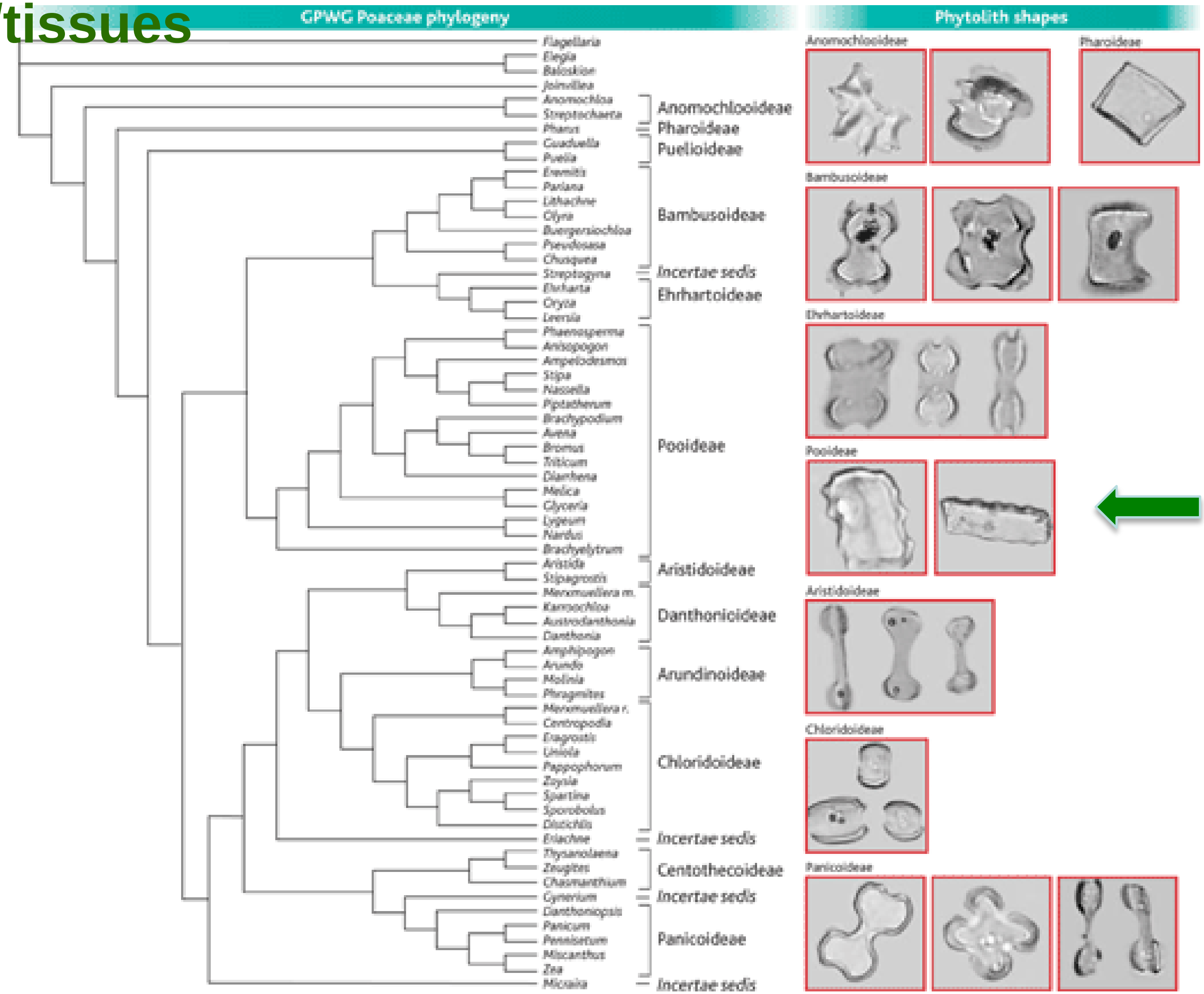
Archeology provides direct evidence, but sometime ambiguous interpretation (fragility is not a perfect criterion).

Ancient DNA evidence: could link between genetics and archeology and solve ambiguous cases, e.g. looking at sequence of fragility gene.



The problem: Most conserved seeds are charred and not suitable for DNA extraction

Phytoliths are silica deposits in plant tissues. They are abundant in archeological sites (in ashes, sediments etc..) Their shape is typical of certain species/tissues



Phytoliths were isolated from modern fresh, modern dry, and ancient sediments

New methods were devised to dissolve silica in conditions that do not damage DNA

ELSEVIER

Quaternary International 193 (2009) 11–19

New methods to isolate organic materials from silicified phytoliths reveal fragmented glycoproteins but no DNA

Rivka Elbaum[a,*], Cathy Melamed-Bessudo[b], Noreen Tuross[c], Avraham A. Levy[b], Steve Weiner[a]

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[c] *Department of Anthropology, Harvard University, 11 Divinity Avenue, Cambridge, MA 02138, USA*

Conclusions part 1:

- **Einkorn wheat: Genetic and Archaeological evidence support domestication in the Karacadag region at the early Neolithic**

- **Barley: Genetic and Archaeological evidence support domestication in the Jordan Valley region at the early Neolithic**

- **Emmer wheat: Genetic and Archaeological evidence are not yet conclusive – must have happened somewhere in the levantine corridor, sometime between PPNA and PPNB (11-10,000 yrs BP)**

- **Domestication was a gradual process over extended periods of mixed culture of wild and domestic types or ? The first domestic types had a phenotype that ressembles the wild wheats (partially fragile) ?**

- **Ancient DNA could solve some ambiguities but is limited by the quality and quantity of the samples**

Possible reasons for the Agricultural Revolution

- **Population pressure and growth of large communities**
- **Reduction in food sources: because of climatic changes (the Younger Dryas)**
- **Spread of the technological breakthrough**

Modifications that occurred during the three phases of wheat cultivation

I. During the transition from wild to cultivated

1. Non-brittle spike
2. Free-threshing spike (naked grains)
3. Non-dormant seeds
4. Uniform and rapid germination
5. Erect plants
6. Increased grain size
7. More spikelets per spike (?)

Modifications that occurred during the three phases of wheat cultivation

II. During 10,000 years of cultivation in polymorphic fields

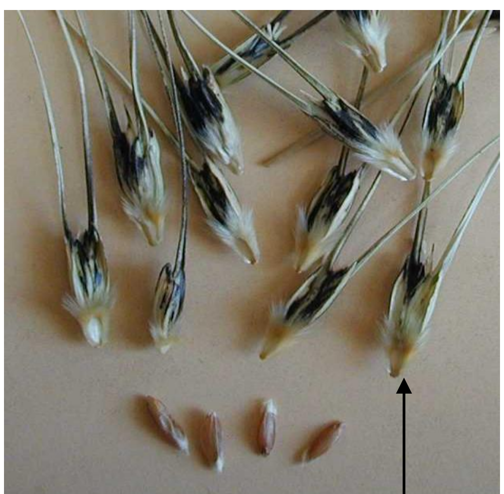
1. Adaptation to new, sometimes extreme, regional environments
2. Increased plant height
3. Development of canopy with wide horizontal leaves
4. Increased competitiveness with other wheat genotypes and weeds
5. Modifications in processes that control phenology
6. Increased grain number per spikelet
7. Improved seed retention (non-shattering)
8. Improved technological properties of grains

Modifications that occurred during the three phases of wheat cultivation

III. During cultivation in monomorphic fields due to modern breeding procedures in the last century

1. Increased yield in densely planted fields; reduced intragenotypic competition
2. Canopy with erect leaves
3. Reduced height
4. Enhanced response to fertilizers and agrochemicals
5. Increased resistance to grain shattering
6. Increased resistance to diseases and pests
7. Lodging resistance
8. Improved harvest index
9. Improved baking and bread-making quality

Wild emmer wheat:
Triticum dicoccoides



Fragile rachis

What genes and what metabolites were affected in the process of domestication?

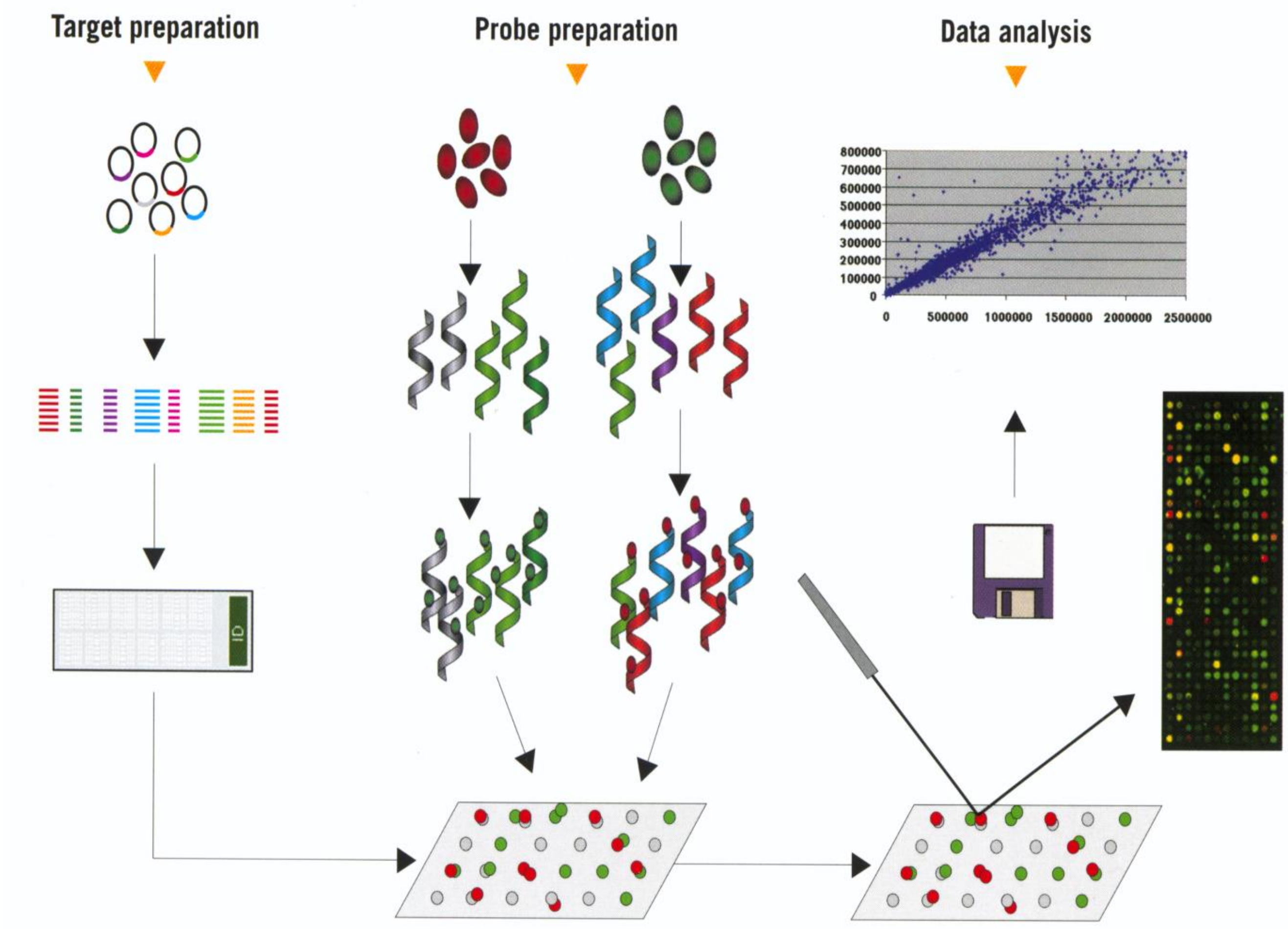
~11,000 years of domesticati

Domesticated wheat:
T. turgidum var duru

Non-fragile rachis

Differential expression of genes and Copy number variation during tetraploid wheat evolution

We used a microarray with 160,000 probes consisting of ~60mer oligos designed for ~40,000 unigenes and 400 transposons



Transcripts that were up-regulated in green tissues of young plants of domesticated wheat

Putative Annotation[b]
caffeic acid O-methyltransferase
phenylalanine ammonia lyase
peroxidase
putative bifunctional nuclease
ribulose 1 5-bisphosphate carboxylase
RuBisCO chain precursor
RuBisCO large subunit
RuBisCO small subunit
sucrose synthase

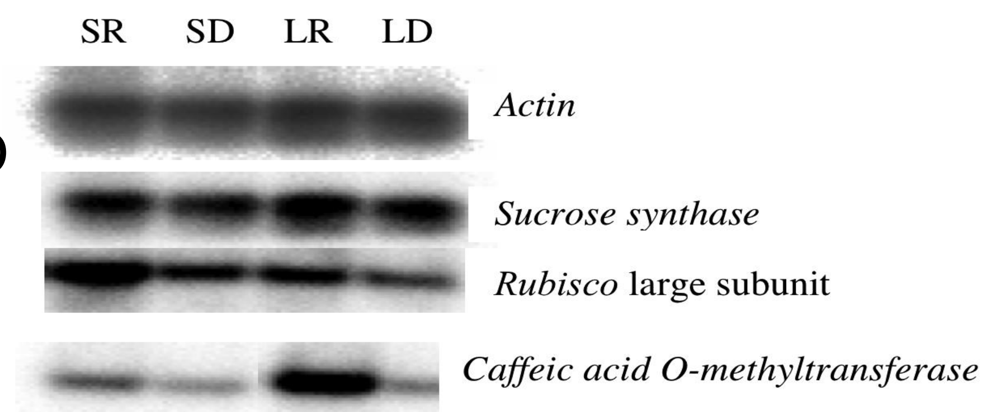
Lignin biosynthesis (strengthened straw)

Carbon metabolism

RT-PCR validation

Leaves dicoccoides=LD

Leaves durum=LR

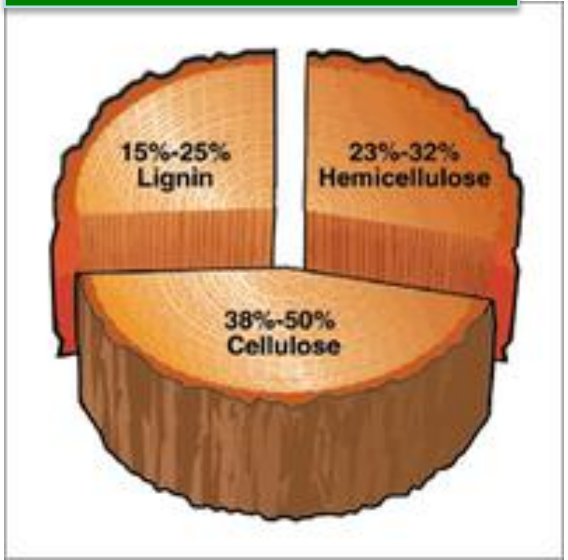


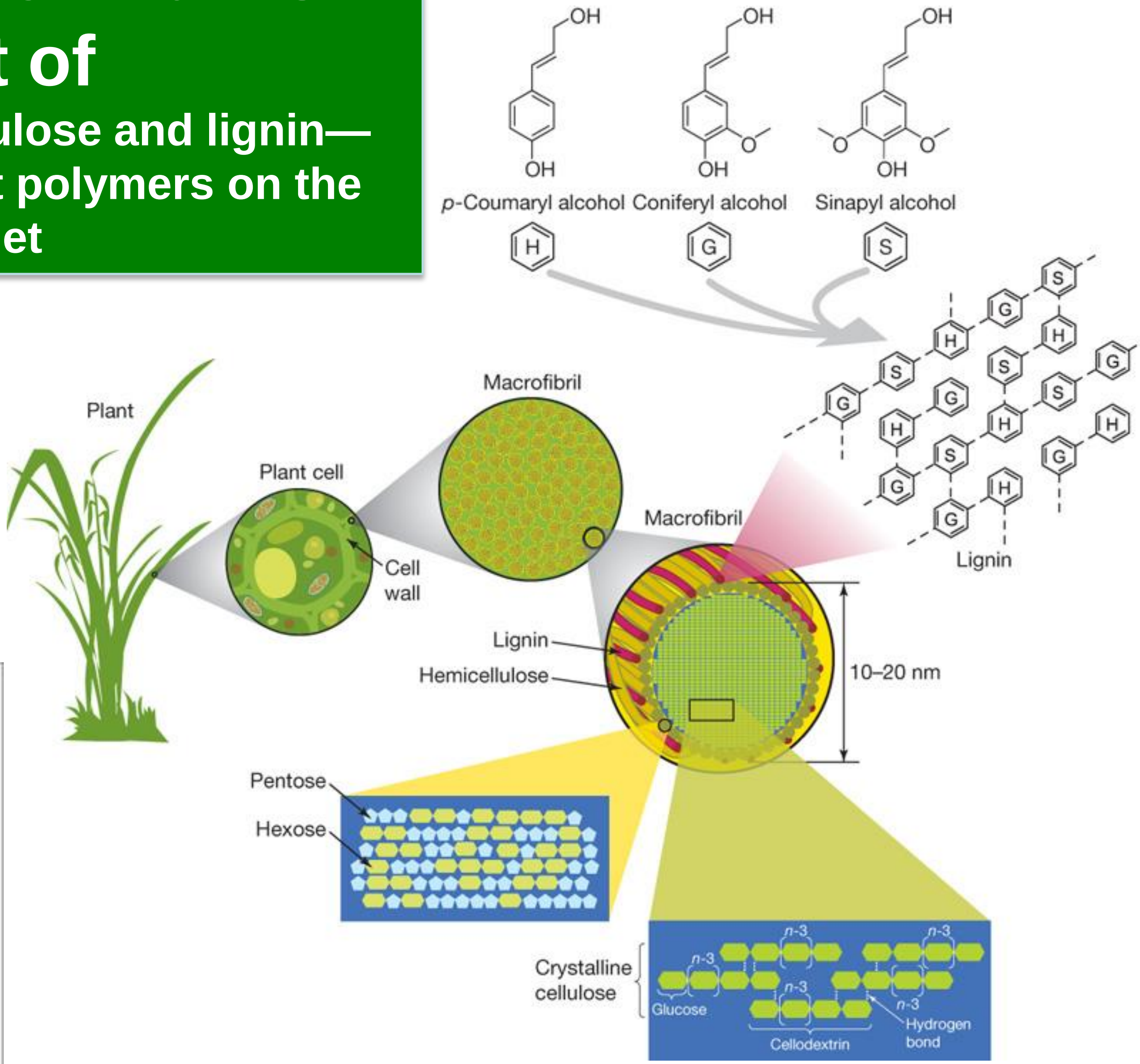
(Sharon Ayal, PhD)

The plant cell wall is built of

Cellulose, hemicellulose and lignin— The most abundant polymers on the planet

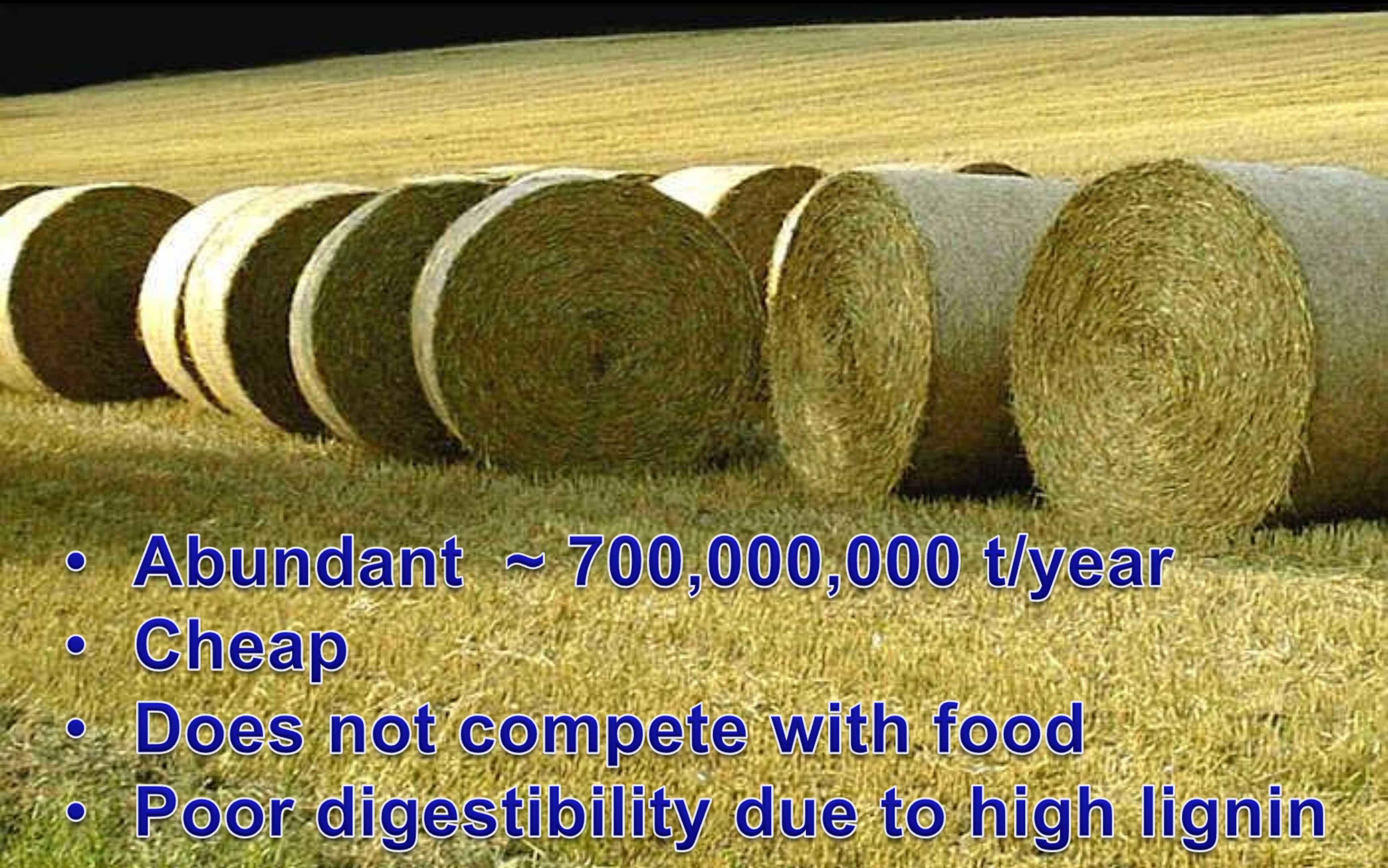
Cellulose and hemicellulose are sources of sugar for fermentation.





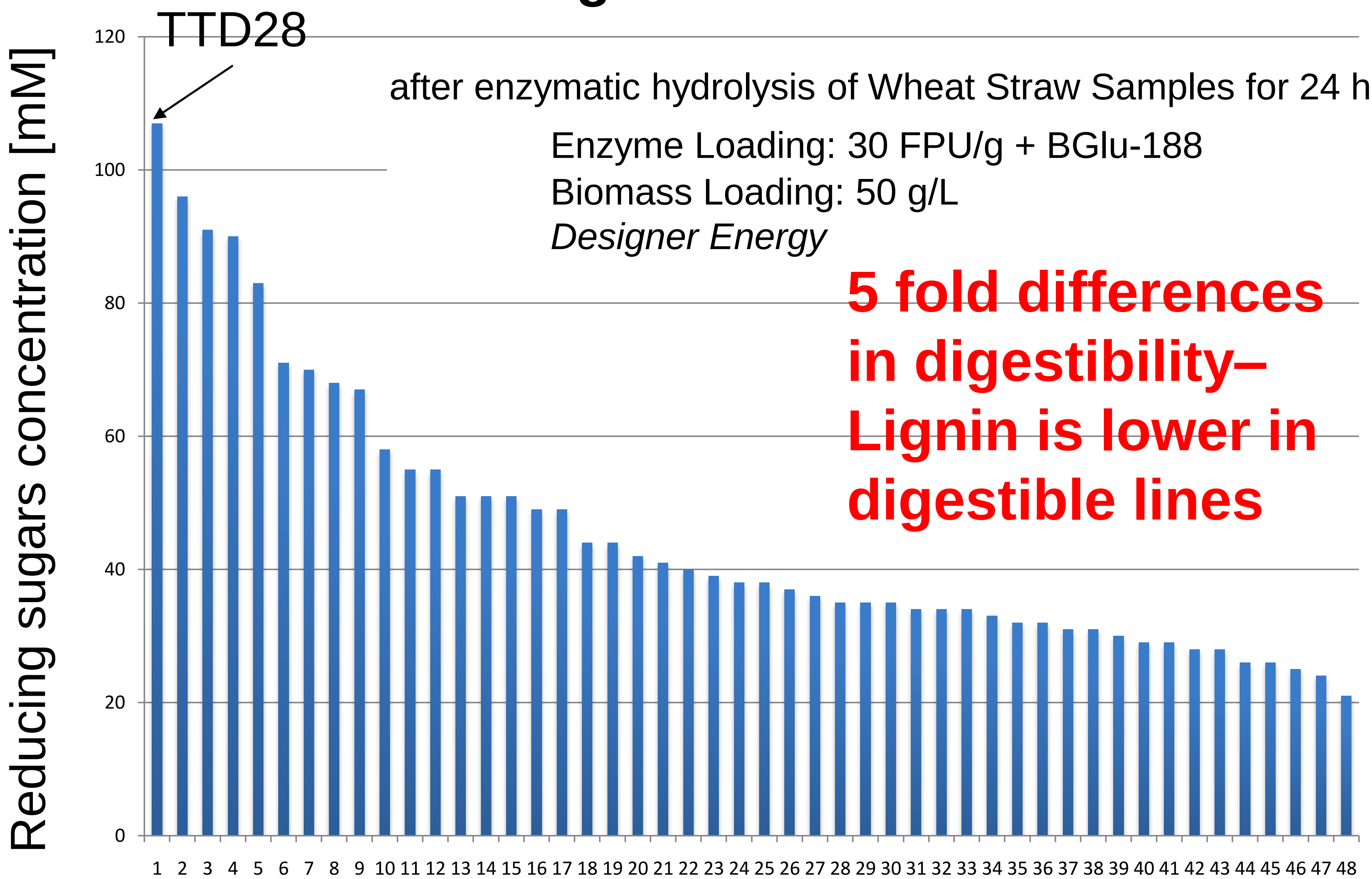
EM Rubin *Nature* **454**, 841-845 (2008) doi:10.1038/nature07190

Wheat straw as feedstock for biofuel



- Abundant ~ 700,000,000 t/year
- Cheap
- Does not compete with food
- Poor digestibility due to high lignin

Straw digestibility without pretreatment among 48 wheat lines



5 fold differences in digestibility– Lignin is lower in digestible lines

Evolution of tetraploid *turgidum* wheat, genome 2n=4x

T. durum

T. dicoccoides
(wild lines)

Fragile, hulled
2 grains/spikelet

→

T. dicoccum
(primitive varieties)

Non-Fragile, hulled
2 grains/spikelet

→

landraces

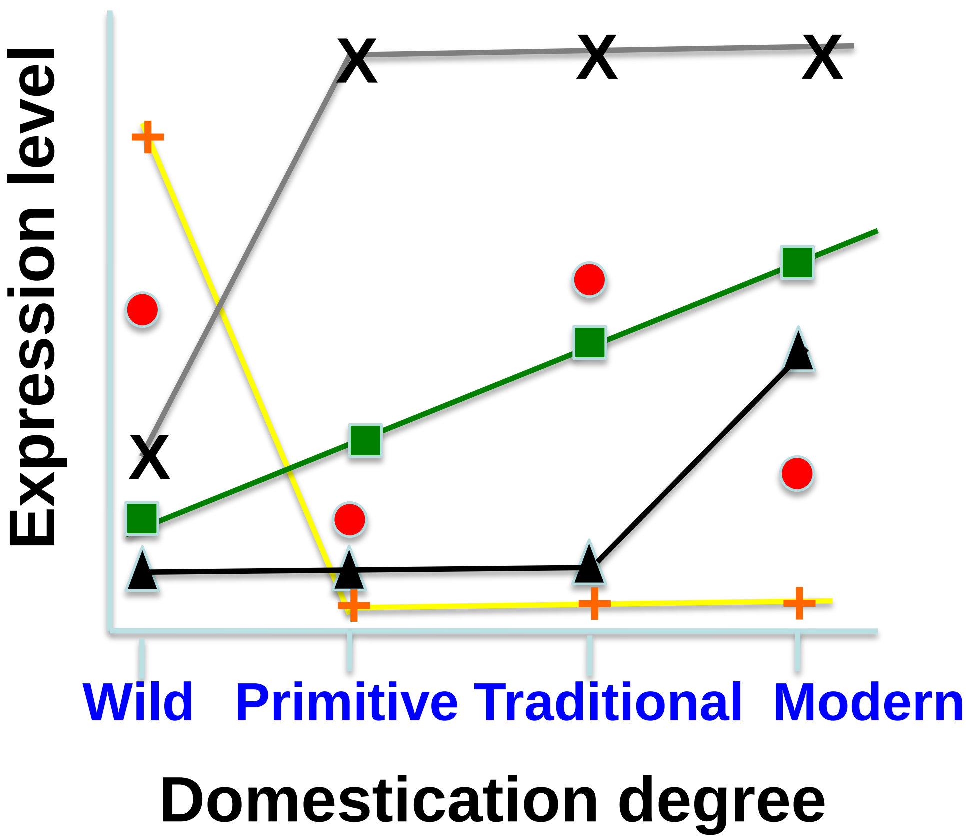
Non-Fragile,
Free threshing
> 2 grains/spikelet

→

Modern varieties

Non-Fragile,
Semi-dwarf
Free threshing
> 2
grains/spikelet

Clustering genes by expression patterns throughout domestication



Genes up-regulated after domestication but not related to high yield in modern wheat: **X**

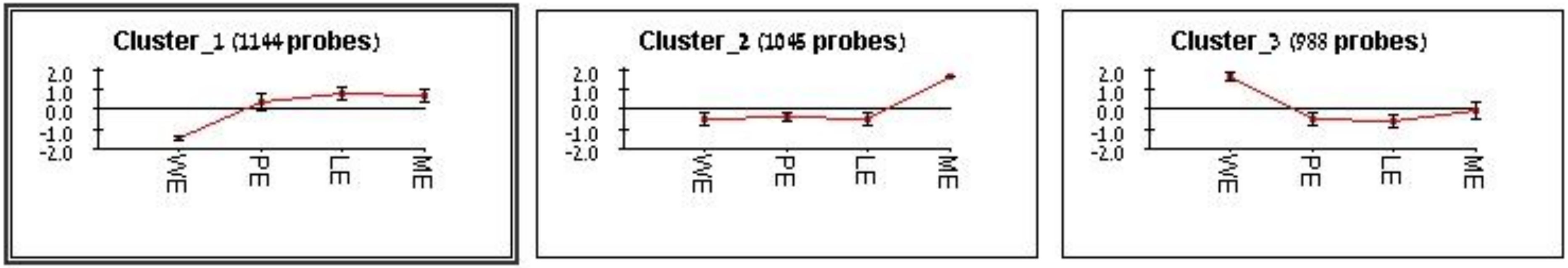
Genes down-regulated (or lost) after domestication: +

Genes correlated with the degree of domestication (■)

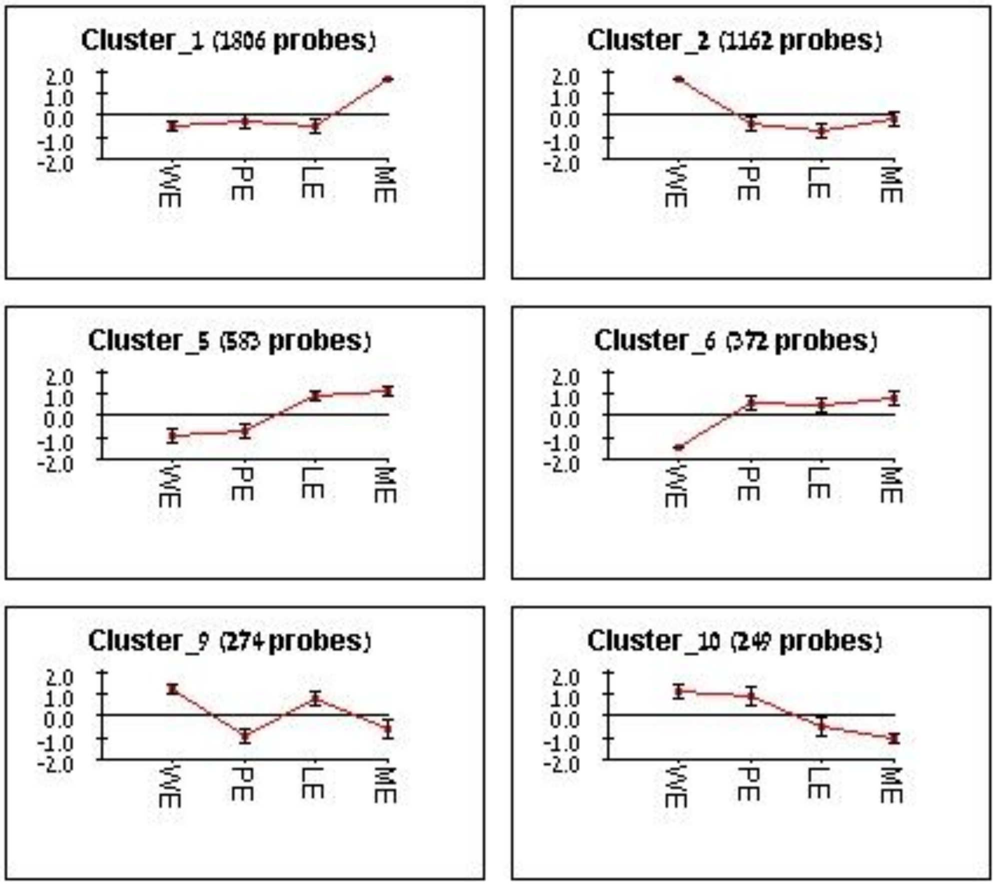
Genes not correlated with domestication but with breeding of modern lines: ▲

Genes with no correlation to domestication: ●

Embryo Transcripts clusters



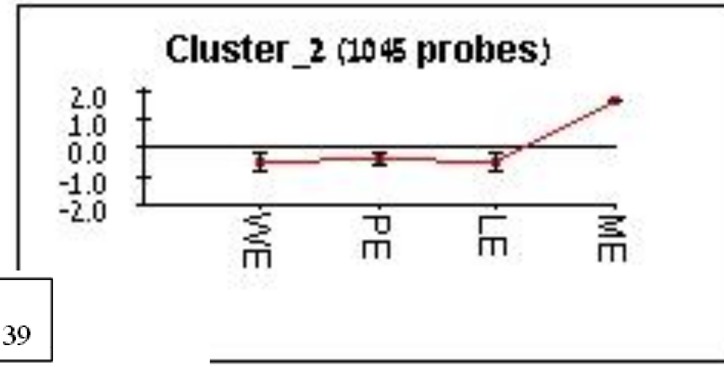
Endosperm Transcripts clusters

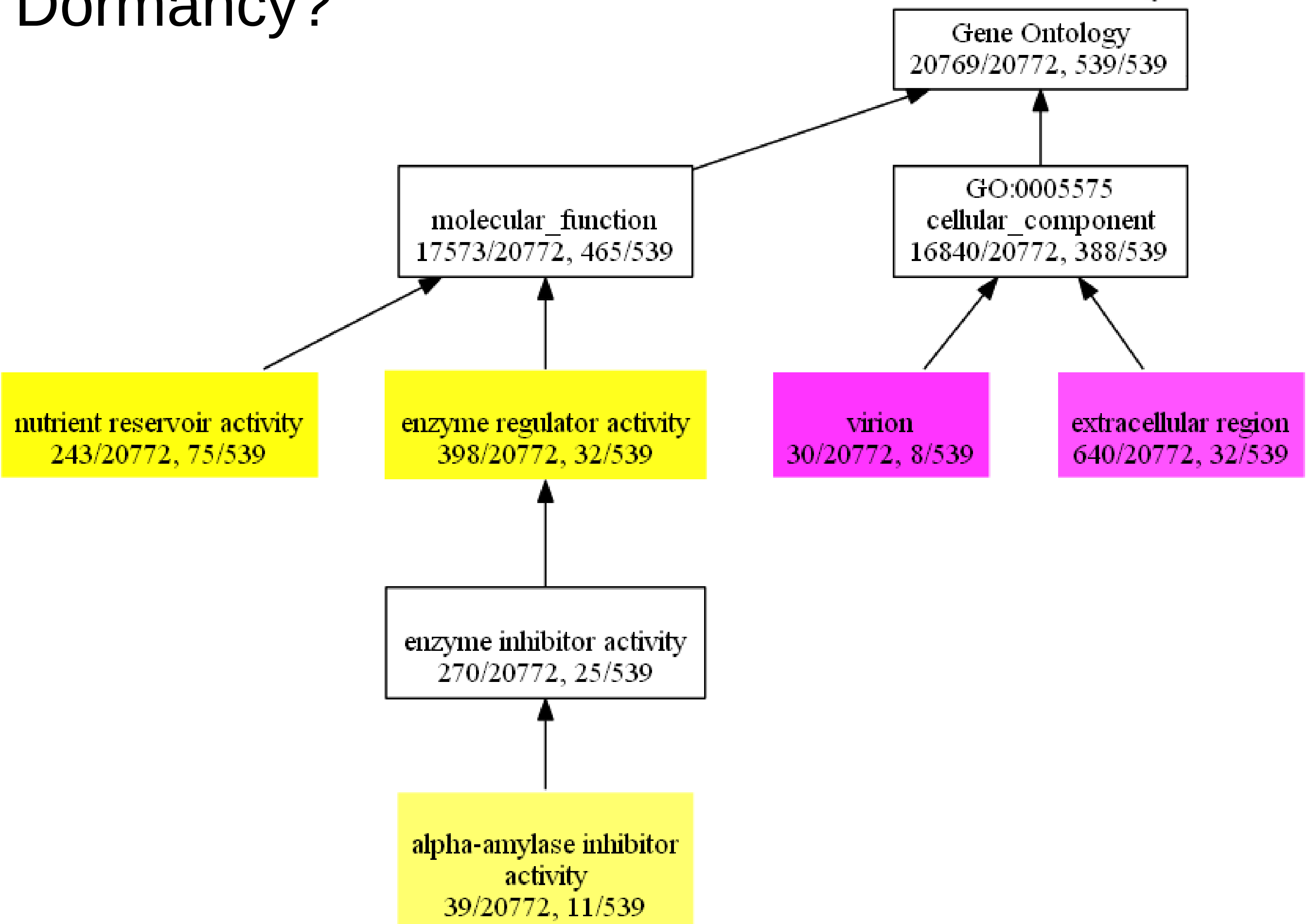


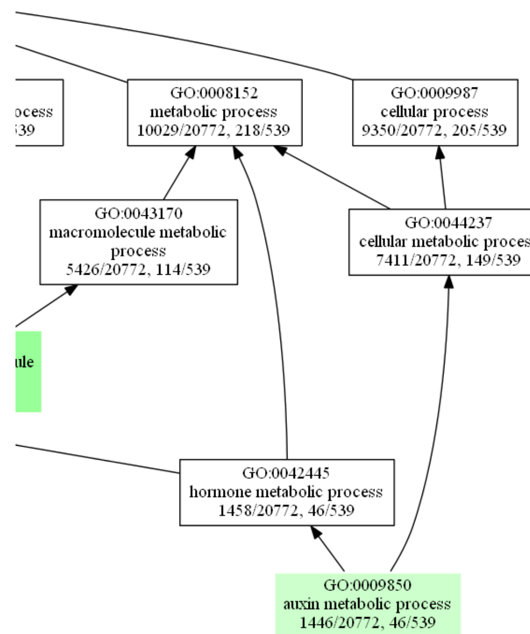
W=Wild
P=Primitive
L=Landrace
M=Modern

Transcripts Embryo Cluster 2-

Genes involved in germination?
Dormancy?

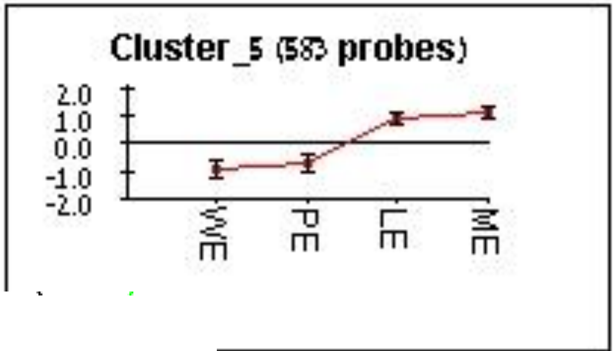


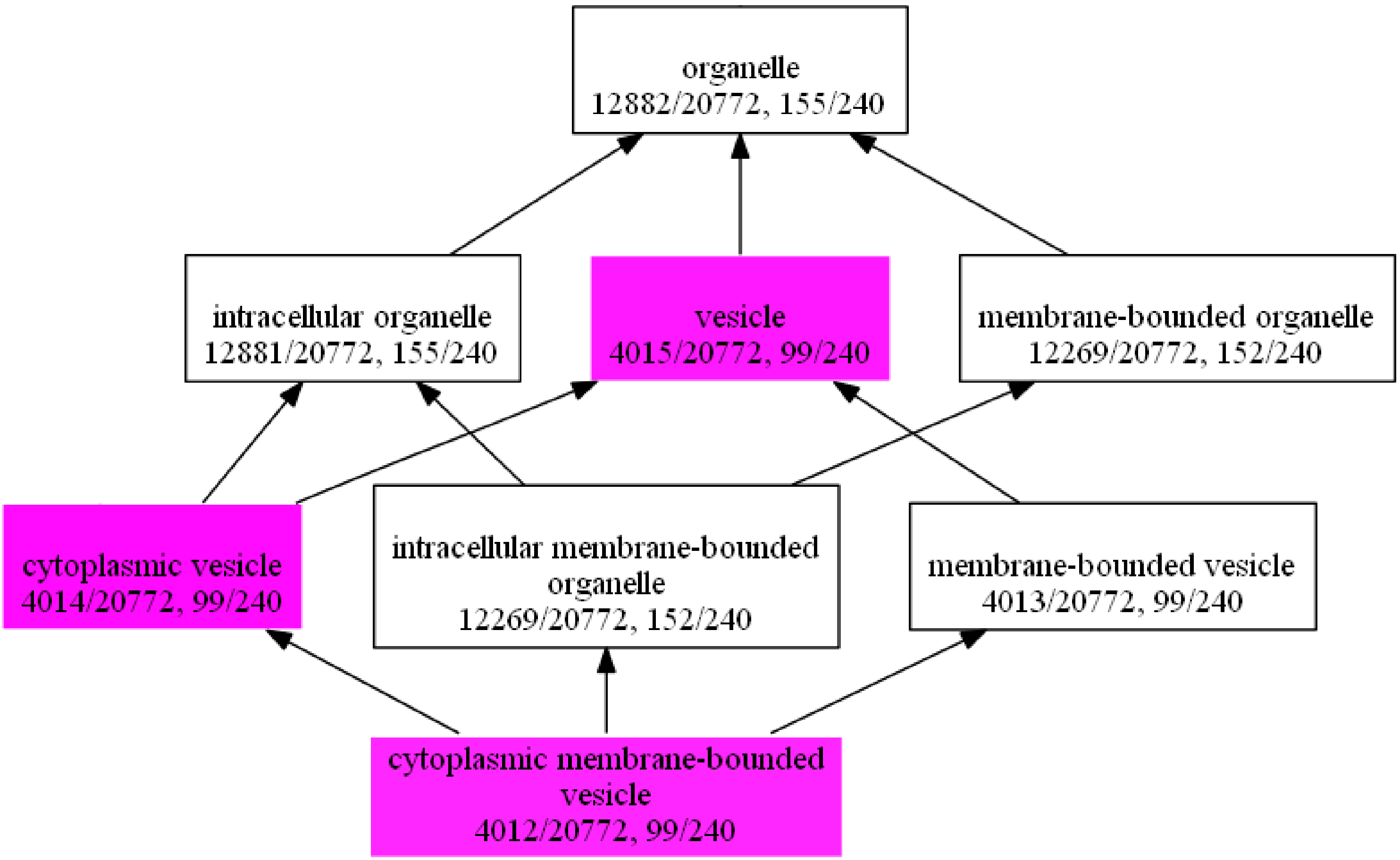




Transcripts Endosperm Cluster 5

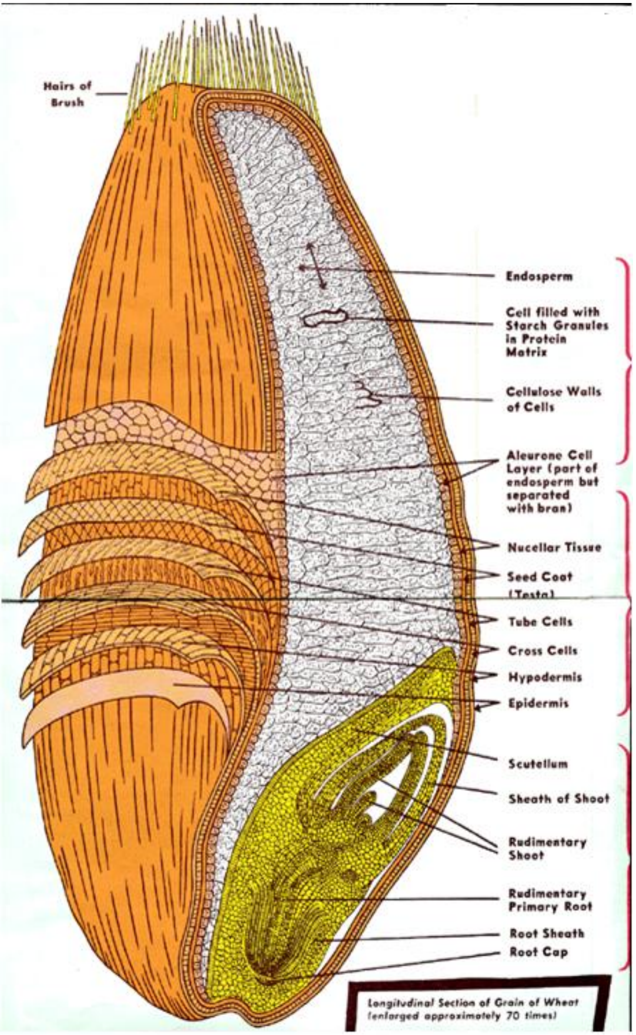
Genes for traficking in the endosperm. Vesicles for protein bodies- Starch or lipids?





What are the changes in metabolite composition that occurred during wheat domestication

(Do we eat the same wheat as our Neolithic ancestors?)



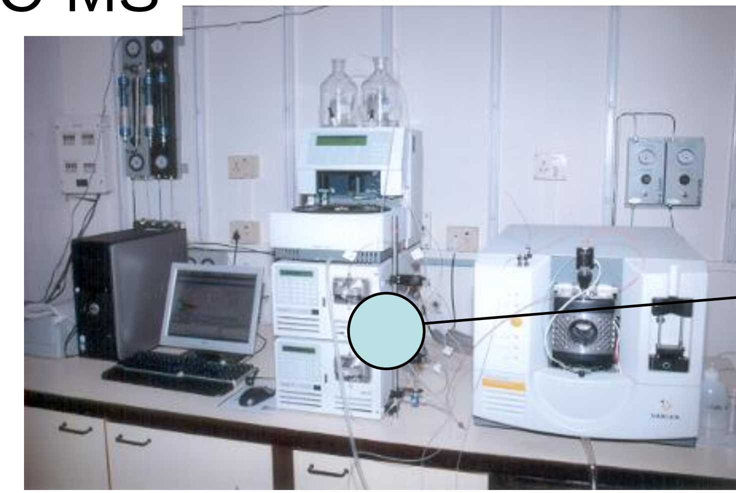
We analyzed secondary and primary metabolites in the endosperm and embryo of wheat grains.

Primary= carbohydrates, lipids, proteins

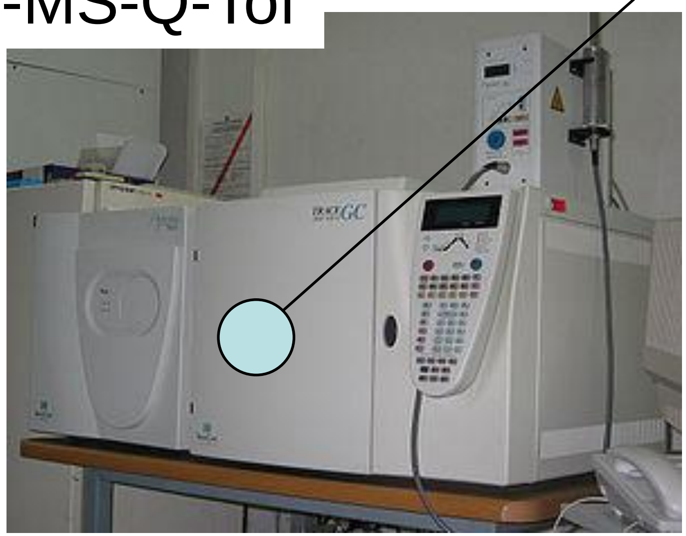
Secondary= alkaloids, phenols, terpenoids etc…

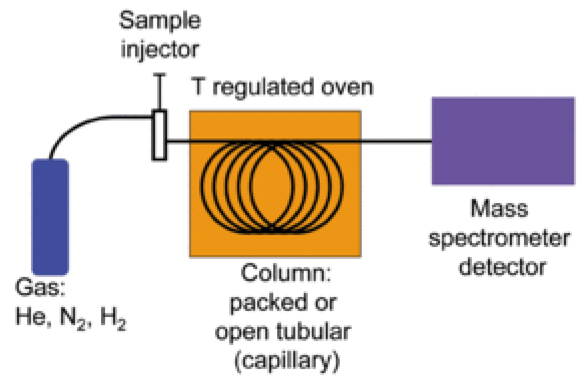
Materials & methods for analytical chemistry:

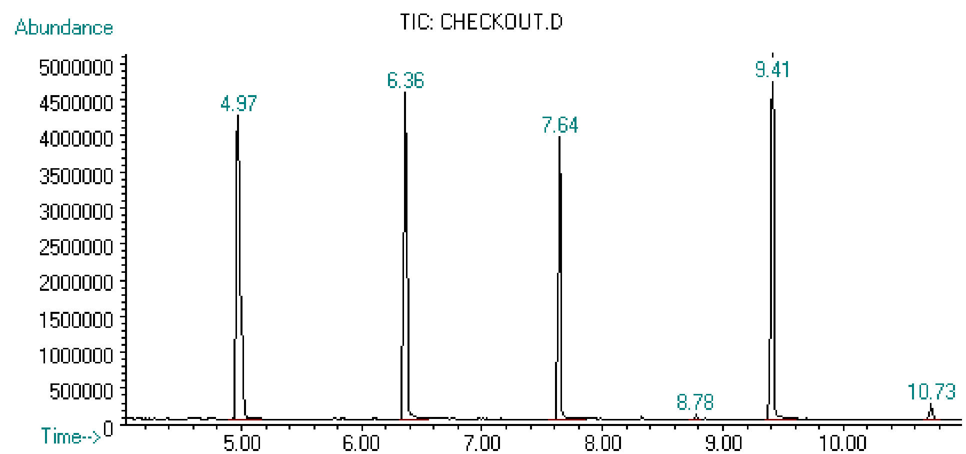
GC-MS



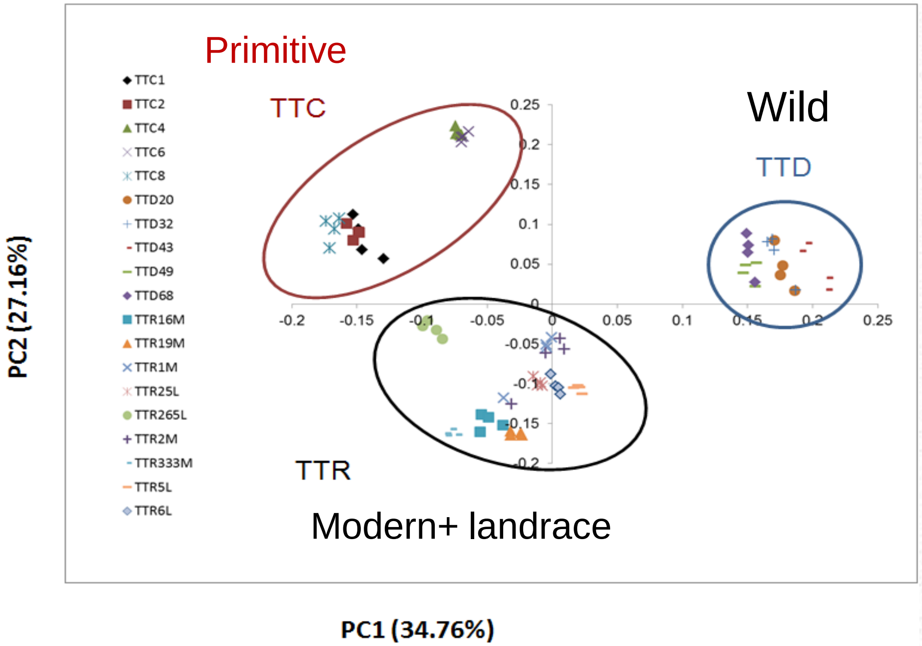
LC-MS-Q-Tof

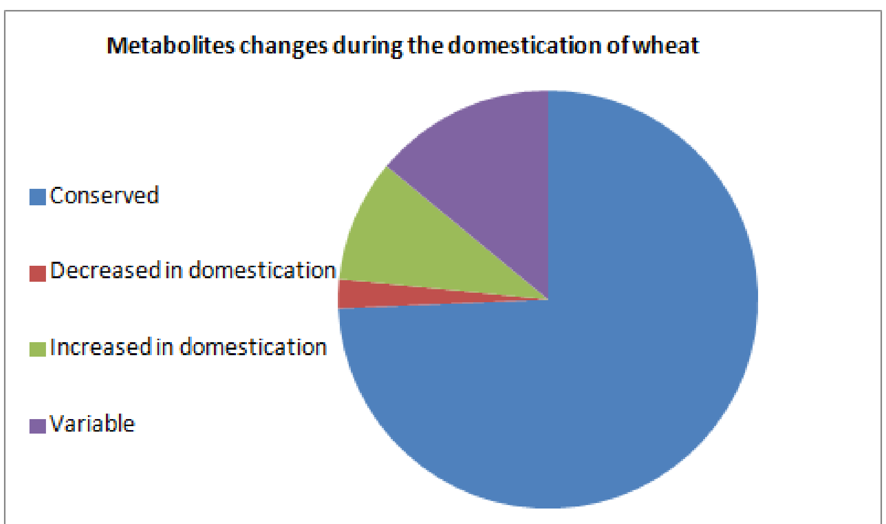


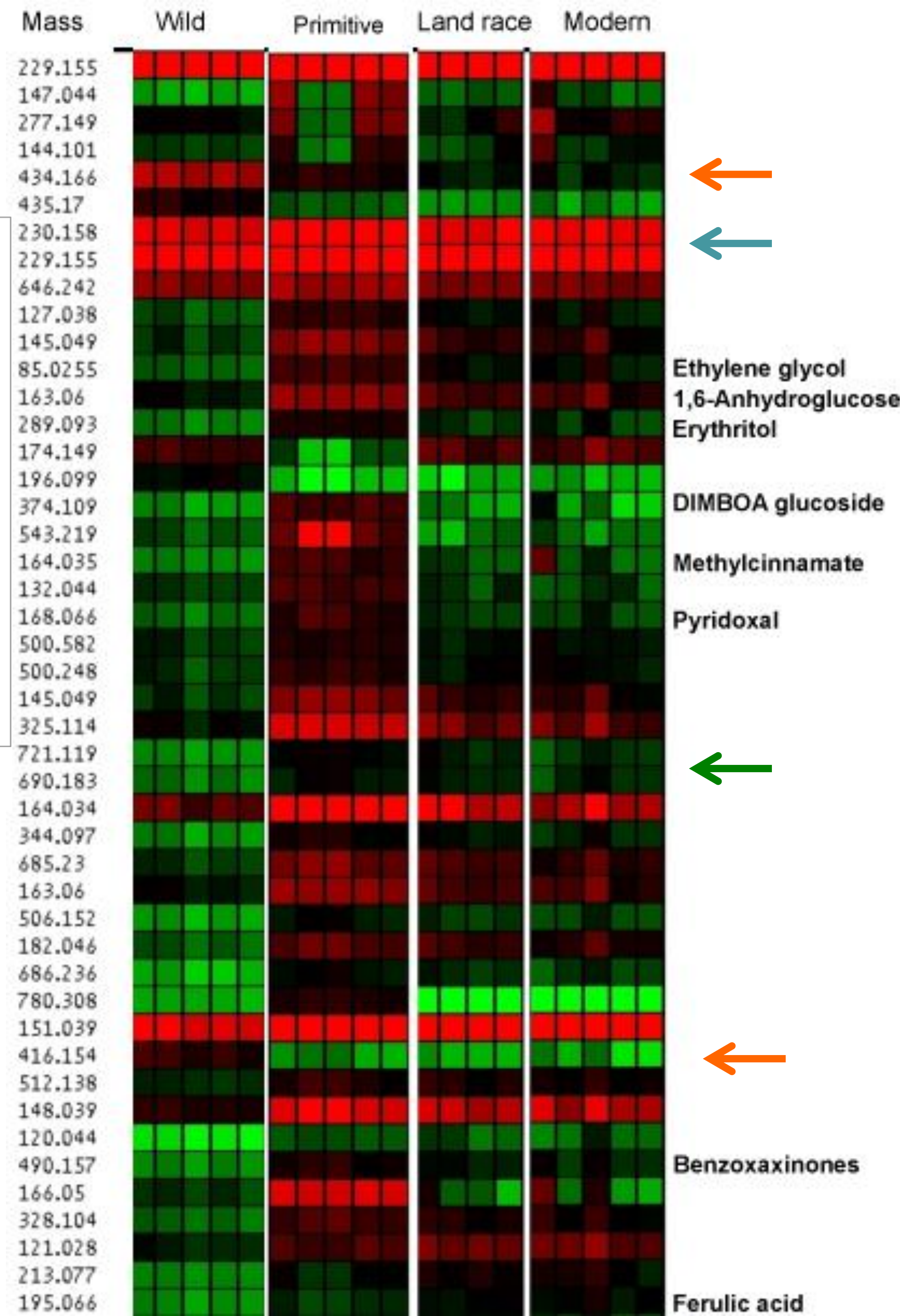




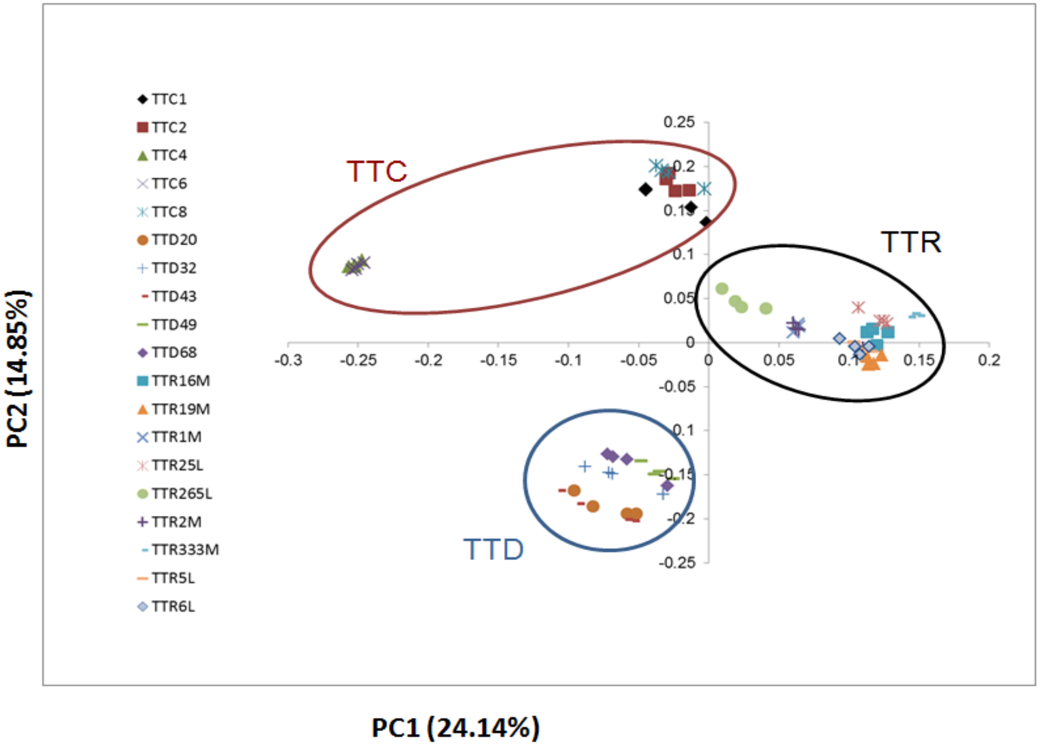
PCA of wheat dry embryo analyzed with UPLC-QTOF-MS in negative mode

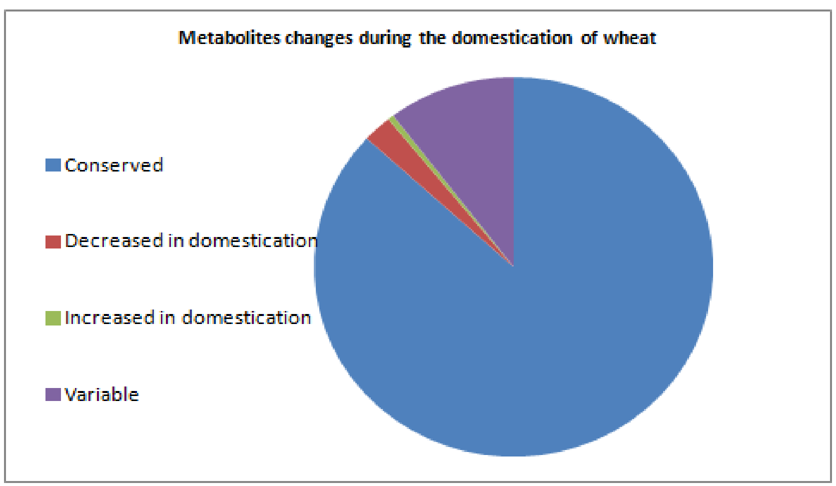


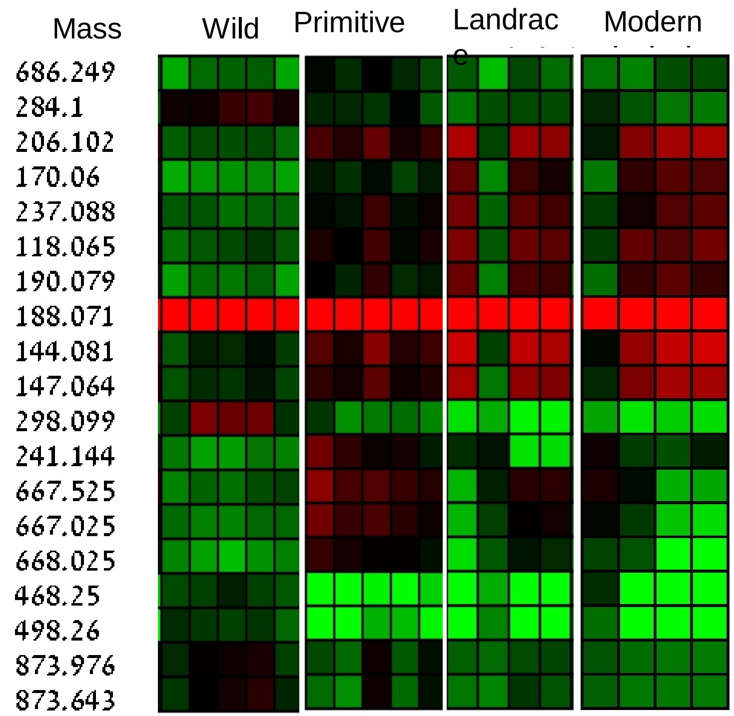




LC-MS on dry endosperm







Conclusions:

Domestication was associated with extensive changes in gene expression, copy number variation, and metabolite composition.

Transcriptome data indicates genes/pathways that were affected by domestication

Genes involved in lignin biosynthesis were up-regulated during wheat evolution, suggesting that wild wheats may enable to improve straw composition for bioethanol.

Metabolic profiling shows distinct patterns for the different evolutionary stages of wheat

Conclusion cont.

The knowledge gained from a study of wheat evolution can be used to continue and strengthen certain trends for yield increase (e.g. carbon fixation)

Conversely, we may want to reverse trends which caused the loss of desirable traits, e.g. specific metabolites, or digestible straw composition

Human selected wheat for a few things they knew and for many things they did not know.

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