History of Agriculture

Prof. Jim Haseloff
http://haseloff.plantsci.cam.ac.uk

NST PMS 1B: Introduction

Prof. Jim Haseloff (jh295@cam.ac.uk)
Links to supplementary lecture materials below:

Lecture 1. Plant breeding and transformation
(Click to see slide show)
(i) Crop breeding: Zea mays as an example, hybrid maize and the rise of agribusiness
(ii) "Ground Zero" for biotechnology
Agrobacterium mediated plant transformation
First plant transformation experiments
Biotechnology in agriculture
(Download Lecture 1 notes in PDF form)

Suggested reading
Biotechnology in the 1930s: the development of hybrid maize. DN Duvick, Nature Reviews Genetics 2:69-73, 2001.
The scientific roots of modern plant biotechnology. IM Sussex, The Plant Cell 20:1189-1198, 2008.
Agrobacterium: nature's genetic engineer. EW Nester, Frontiers in Plant Science 5:1-16, 2015.

Lecture 2. Genetics and phenotype
(Click to see slide show)
(i) Gene design
(ii) Single gene traits
(iii) Reporter genes
(iv) Visualising cell architecture
(Download Lecture 2 notes in PDF form)

Suggested reading
Towards two decades of plant biotechnology: successes, failures and prospects. N Halford Food and Energy Security 1:9-28, 2012.
GM plants: questions and answers. Royal Society Report, 2016
Using intrinsically fluorescent proteins for plant cell imaging. R Dixit, R Cyr and S Gilroy, The Plant Journal 45:599-615, 2006.

Lecture 3. Plant biotechnology
(Click to see slide show)
(i) Genome scale DNA engineering and precision breeding
(ii) Synthetic Biology
(iii) Global challenges
(iv) Problems and technical opportunities.
(Download Lecture 2 notes in PDF form)

Suggested reading
Convergent traits
Shatterproof genetics
Bayer trait development story

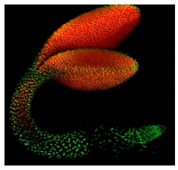
Following lectures: CO2 levels, photosynthesis and carbon capture (Hibberd); Nutrient availability (Davies); Global warming: Drought and water relations (Griffiths); Temperature responses (Tanner)

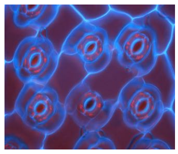
Part 1B Practical Class
Plant transformation and use of reporter genes and microscopy of plants.
In this practical session there are several objectives: (i) to describe gene fusions, (ii) the use of reporter genes in plants, and (iii) the use of microscopy techniques for reporter gene detection in the context of plant transformation.
These pages contain copies of images used in the practical, and articles for further reading are provided as downloadable PDFs (Click on the menu items to access these).

1

Course Aims and Philosophy

- Provide an integrated overview of plant and microbial biology
- Address all levels from molecules to ecological communities





2

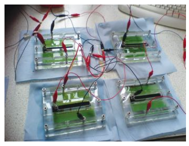
Lecture Content Overview

- Fundamental aspects of plant biology and microbiology
- Related to current world issues e.g.
  - Biofuels
  - Crop protection
  - Climate change

3

Practical Classes I

- Brand new teaching lab
- Integrated practicals:
  - Make your own GM plant containing a reporter gene
  - Physiology of tobacco with Rubisco antisense constructs
  - Plant Pathology



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Practical Classes II

Visits to:

- Botanical Garden
- NIAB Innovation Farm
- Local Field Sites (e.g. Hayley Wood)

5

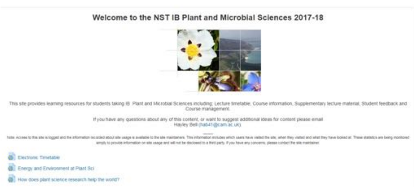
Portugal Field Trip

- Mini projects
- See lecture material out in the field
- Sunshine!
- 18th March-25th March 2018
- Sign up on Moodle

6

Support on Moodle

- Lecture and practical material
- Glossaries for every lecture block
- Interactive resources for consolidation



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8

NST PMS 1B: Origins of modern agriculture

Prof. Jim Haseloff (jh295): Supplementary lecture materials at **haseloff.plantsci.cam.ac.uk**

L1. **Plant breeding and transformation**
(i) Crop breeding: *Zea mays* as an example, hybrid maize and the rise of agribusiness
(ii) "Ground Zero" for biotechnology
- Agrobacterium mediated plant transformation
- First plant transformation experiments
- Biotechnology in agriculture

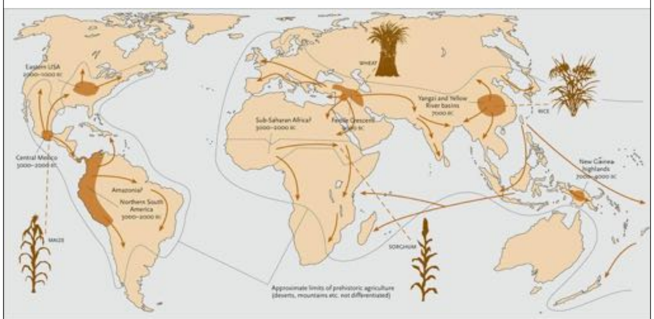
L2. **Genetics and phenotype**
(i) Single gene traits
(ii) Reporter genes, regulatory networks and multicellularity
(iii) Physiology and morphogenesis
(iv) Diversity and trait development

L3. **Plant biotechnology**
(i) Genome scale DNA engineering and precision breeding
(ii) Synthetic Biology
(iii) Global challenges
(iv) Problems and technical opportunities.

Following lectures: CO_2 levels, photosynthesis and carbon capture (Hibberd); Nutrient availability (Davies); Global warming: Drought and water relations (Griffiths); Temperature responses (Tanner)

9

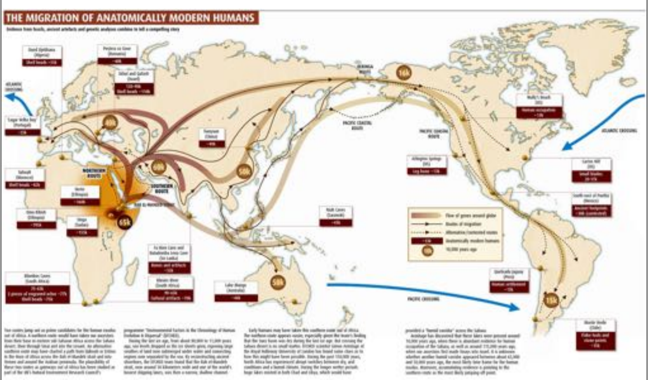
Origins of world crops



Nikolai Vavilov

10

Nicola Vavilov was a Russian biologist who first popularised the idea of geographical centres of diversity for the origin of modern crop species. These centres corresponded to areas of botanical diversity that coincided with the establishment of early human societies and plant domestication.

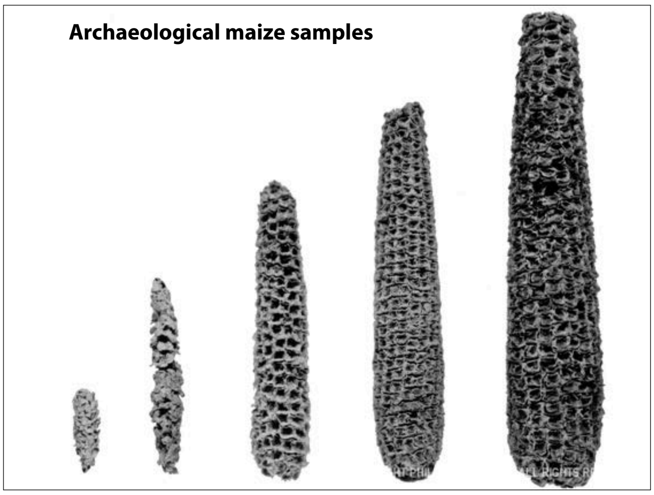


Human migration and establishment of population centres

11

Current theories for the evolution of anatomically modern humans, include origin in west Africa and successive waves of migration into Europe, Asia and the Americas - starting over 65,000 years ago. By 15,000 years ago modern humans had reached Mesoamerica. Over the following millennia local people shifted from a nomadic lifestyle to an existence based on agriculture, and began the domestication of local plant species. In this lecture we follow the history of human use for one of these plant species, maize.

12 Diorama at the American Museum of Natural History showing an Aztec market in Tenochtitlán, the capital city of the Aztec empire in ancient Mexico - in the year 1519, immediately prior to the arrival of Europeans. By this stage maize had been grown and selected for around 7000 years, and could be found in recognisably modern form.



13 Corn cobs, Zea mays; plant remains; Tehuacan Valley, Puebla, Mexico; c. 5000 BC to AD 1500. Archaeological excavations have revealed a series of intermediate forms of maize, and these have been dated and can be arranged on a timeline - with cobs ranging from small vestigial forms through to large modern forms due to selective propagation of seed. Robert S. Peabody Museum of Archaeology, Phillips Academy, Andover, Massachusetts.

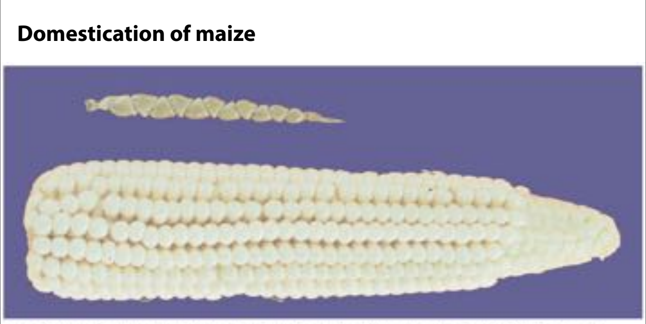
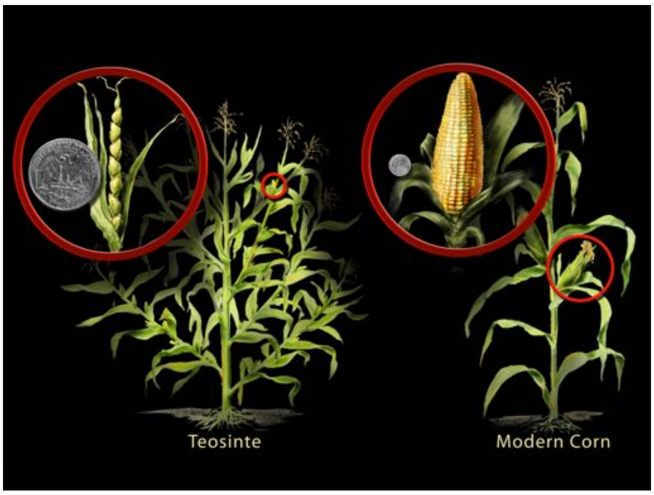
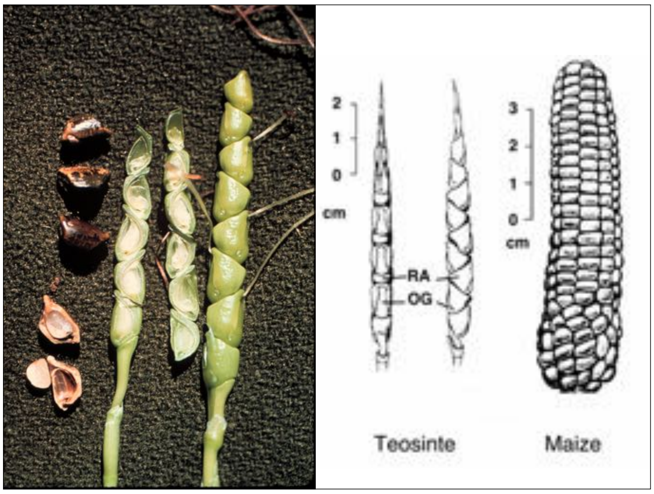


Figure 1. Domestication of corn. The upper image shows the mature inflorescence, or "ear" of teosinte (*Zea mays* ssp. *mexicana*), the probable wild progenitor of modern corn (or maize, *Zea mays* ssp. *mays* L.), shown in the lower image. The teosinte inflorescence has no cob, allowing the seed to separate and disperse easily when they are mature. Selection over time by early agriculturalists resulted in types that retained their seed on the ear, leading to the development of the cob. Modern breeding has greatly increased the size and number of seed per ear. (Courtesy J. Doebley, University of Wisconsin)

14 Early forms of Maize strongly resemble Teosinte, a plant endemic to Mesoamerica, and a subspecies of Zea mays. this likely progenitor has a strikingly distinct morphology, with smaller numbers of kernels arranged on a spike. It is estimated that new varieties of maize been selected for over 7000 years. Modern varieties are characterised by a cob architecture with much larger numbers of kernels on each inflorescence.



15 The overall habits of Teosinte and modern maize plants are strikingly different. Teosinte plants are more highly branched with multiple male and female inflorescences. Graphical representations are shown with a coin added for scale. Modern maize plants are taller with a higher degree of apical dominance, and are better adapted for modern agricultural practices.



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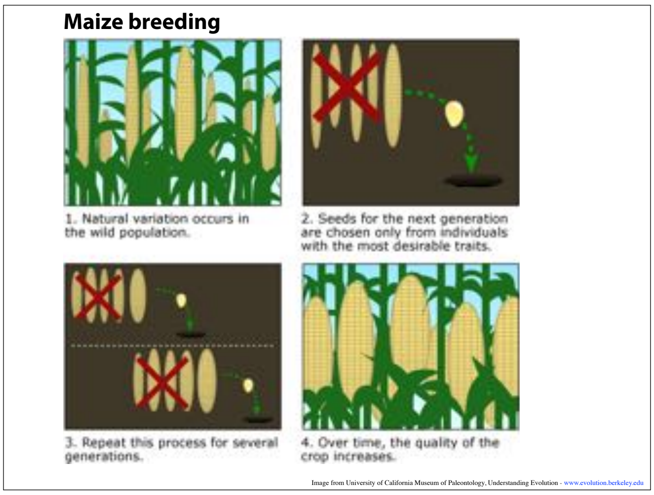
A close-up view of teosinte, showing the hardened integument that normally covers the seed. Harvested Teosinte seed need to be broken open to release the nutritious kernel. The selection of improved varieties over millennia produced maize varieties that lacked this integument.



17

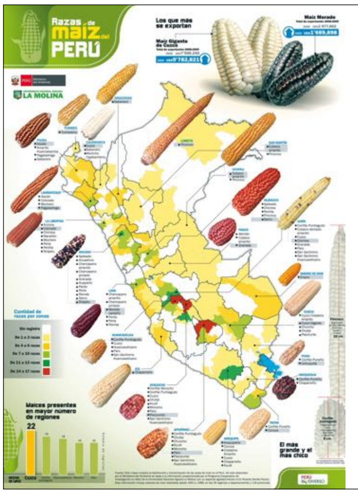
Wild Teosinte and primitive maize varieties are self fertilising and true breeding plants. Genetic variation occurs naturally in these populations. Early agriculturalists simply chose plants with improved characters and selectively planted these seed. the cumulative effect of this simple selection procedure over many generations gave rise to plant lines with many improved characters.

18

Maize rapidly became a staple crop and took on greater, even religious significance. The application of crop selection gave rise to a myriad of new maize varieties.
Chicomecoatl, was the Aztec goddess of maize, agriculture and fertility. Chicomecoatl was the deity who ensured that maize kernels turned into thriving plants. When plants began to sprout, the Mexica held special celebrations where young women let down their hair and danced through the fields. They each picked five ears of corn, wrapped them up as if they were infants, and danced them back from the fields in a great procession full of music. As part of the festivities, people would douse each other with flower pollen or scented maize flour. After harvest, a young girl dressed as Chicomecoatl was sacrificed by decapitation and her blood was collected so that it could be poured over a statue of the goddess. The priests then flayed her corpse and wore the skin. https://en.wikipedia.org/wiki/Centeotl

19

New maize varieties with highly individual characteristics were selected and maintained across Mesoamerica. Farmers developed local varieties with different characteristics. Some examples are shown here. Traditional respect for the different properties of these local corn varieties has resulted in the historical maintenance of a large number of races or varieties. Today, the conservation of diversity in maize has been taken up by international seed banks such as CIMMYT (http://www.cimmyt.org)



Maize diversity spread across South and North America

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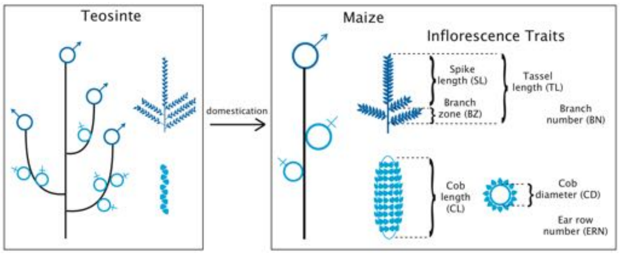
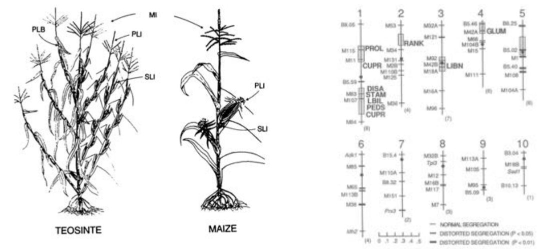
Diverse true breeding populations of maize have been found widely across South America and North America, as a result of the historical spread of maize as a staple crop. This poster shows the distribution of distinct varieties of maize across local agricultural regions in Peru.



Maize domestication was accompanied by modification of many plant traits related to agronomy, growth and yield

21

One can take a more analytical approach to describe the changes that occurred during domestication of maize. Here, formal annotation is used to describe the inflorescences and branching of Teosinte and maize. Domestication saw the origin of a series of novel characters that corresponded to modification of the original plant morphology. These include creation of branching patterns and arrangement of female flowers and therefore kernels.



Major differences between maize and teosinte map to few loci

Table 1. List of principal traits distinguishing maize and teosinte

Trait	Description
CUPR (cupules per rank)	Number of cupules in a single rank
DISA (disarticulation score)	Tendency of ear to shatter (1–10 scale)
GLUM (glume score)	Hardness and angle of outer glume (1–10 scale)
LBIL (lateral branch internode)	Average length of internodes on the primary lateral branch
LIBN (branch number)	Number of branches in primary lateral inflorescence
PEDS (pedicellate spikelet score)	Percentage of cupules lacking the pedicellate spikelet
PROL (prolificacy)	Number of ears on the primary lateral branch
RANK (rank)	Number of rows of cupules
STAM (staminate score)	Percentage of male spikelets in primary lateral inflorescence

Doebley *et al.*, PNAS (USA) 87: 9888-9892 (1990)

22

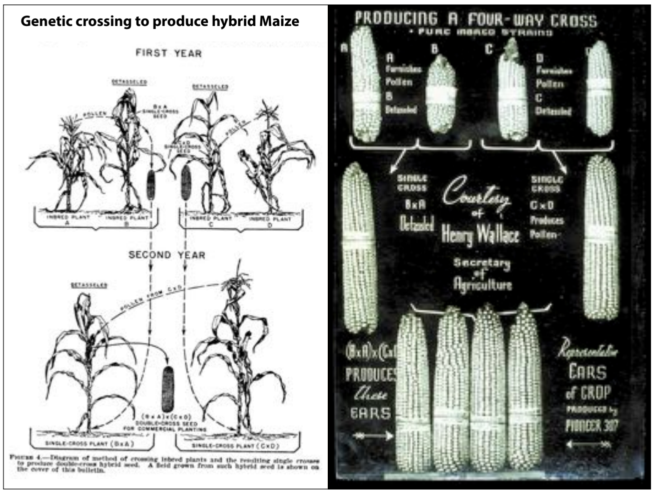
Work from John Doebley's lab has mapped the genetic differences between Teosinte and maize. genetic mapping studies have identified genes known to be involved in vegetative branching, morphology and floral architecture. Strikingly it was estimated that around 90% of the difference in form between Teosinte and maize could be accounted for by less than ten genetic loci.

Maize farming in the US Midwest circa 1900

23

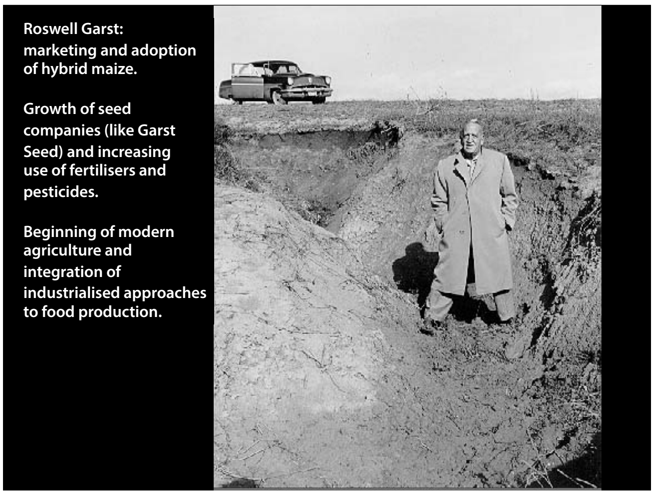
Europeans adopted maize as a crop and the 1800s saw large plantings across the Midwest of the United States. Before 1900 farmers in the Midwest were highly self-sufficient. They looked to the outside world for things like salt and nails, but external inputs into crops were minimal. Fertiliser inputs were limited to manure, pesticides were unknown and crops were true breeding and seed corn was obtained from previous year’s crop. County fairs included competitions for the highest yielding corn plants.

24



In the 1900s scientists like GH Shull observed that open pollinated inbred forms of maize became less productive over time. In contrast heterosis or out-crossing gave rise to highly productive progeny. (Maize plants have separate male and female flowers and detasseling of male flowers is a simple way of ensuring selective crossing). Through the 1920s, plant breeding stations were established to create parental inbred lines that could be used for different crosses and to create highly productive maize seed.

25



Entrepreneurs like Roswell Garst helped transform US agriculture last century. He helped to establish sales of hybrid corn seed with the noted corn breeder Henry Wallace in 1930s in Iowa. Wallace established Pioneer Hi-bred, and Garst established Garst seed. Farmers were previously highly self reliant - saving a portion of their crop for next year's seed, using manure for fertiliser, and using draft horses for ploughing and carting the hand-picked corn. Garst offered free bags of hybrid seed corn in return for half of the next seasons increased yield. When the new seed outperformed, he only accepted the cost of the seed corn - in return for a commitment for the following season. Farmers soon switched to purchasing seed corn for cash.
Eventually this led to the conversion of farming from an occupation, to an industry. There was a loss of diversity, from 786 varieties in 1903 to 52 in 1983 - and increased application of synthetic fertilisers, pesticides and herbicides. Machinery was invented for handling of the more uniform crops. Integration of these activities gave rise to agribusiness.

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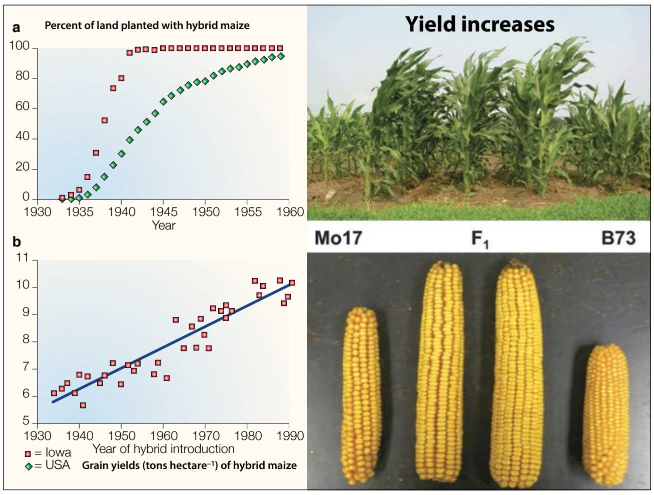
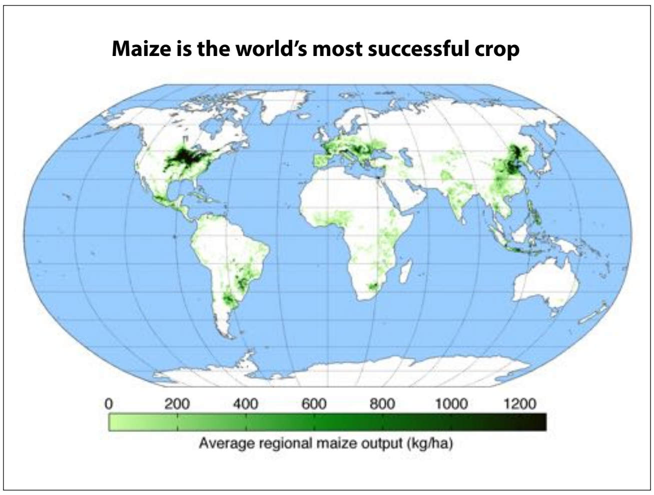
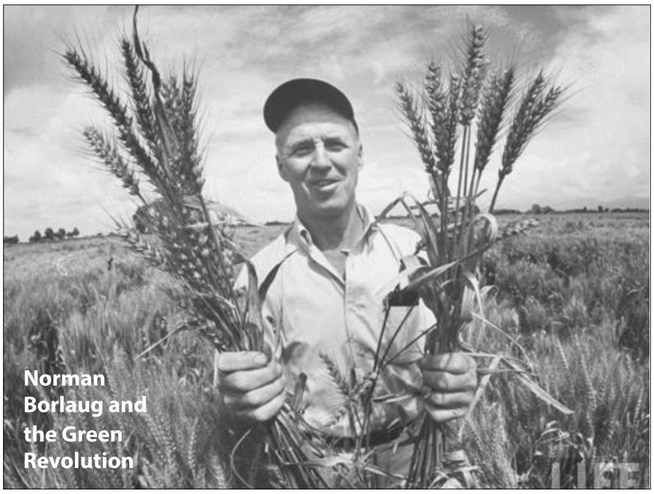


Hybrid maize seed saw rapid adoption in the US Midwest after its introduction in the 1930s. the overall percent plan planted with hybrid maize increased rapidly. In addition, new varieties of hybrid maize saw rapid increases in productivity over the coming decades. Photographs are shown of parental lines and hybrid progeny.

27



From its origin as a Mexican weed, worldwide production of maize is over 1 gigatonne per annum, more than wheat or rice. (http://www.fao.org/faostat/, and http://www.worldofcorn.com). The USA and China are the major producers of maize.



28

Selective breeding of other crops has dramatically improved their yields also. The decades following 1960's saw the breeding of highly productive new varieties of wheat. Many of these varieties were dwarf, which provided agronomic benefits and allowed commitment of more resources to seed production during growth. In addition, improved response to inorganic fertilisers and introduction of disease resistance through cycles of out-crossing and back-crossing contributed to new elite varieties.

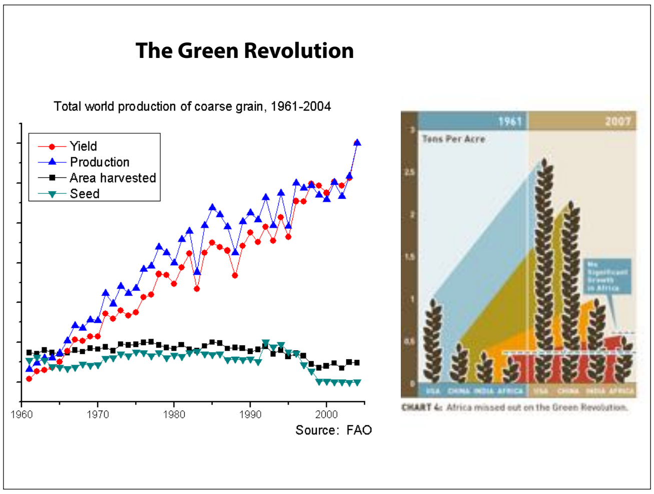
Norman Borlaug was a pioneer of these efforts. He is shown here with Sonora-64, one of the semi-dwarf, high-yield, disease-resistant varieties that was key to the Green Revolution, to a group of young international trainees, at what is now CIMMYT's CENEB station (Campo Experimental Norman E. Borlaug, or The Norman E. Borlaug Experiment Station), near Ciudad Obregón, Sonora, northern Mexico.

29

The harvesters by Pieter Bruegel the Elder (1565) - with a graphic representation of harvested wheat field in northern Europe. Note that the height of these wheat crops reached shoulder height.

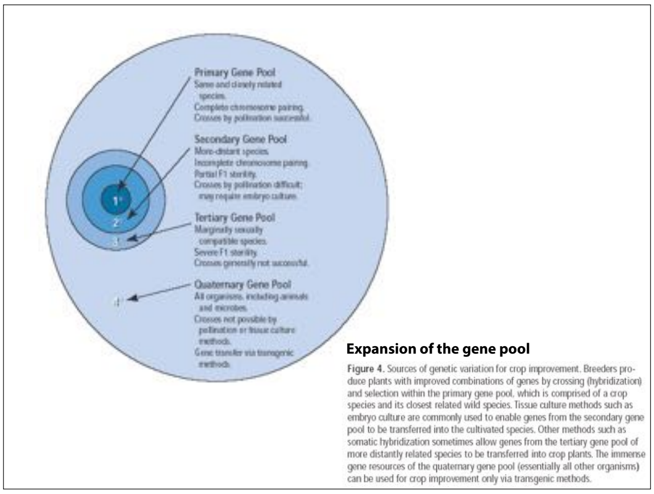
30

Modern wheat crops are much shorter, shown here with Norman Borlaug and colleagues at a trial field of Sonora-64.
The story of Borlaug career is inspiring, a short version can be found at https://en.wikipedia.org/wiki/Norman_Borlaug. He has been credited with saving a billion people from starvation, and his work has been extended to rice varieties.



31

From the 1960s the worldwide production of grain has increased dramatically in yield and total production despite relatively constant area of cultivation and planted seed. The bulk of these increases have been seen in the developed world, China and India. The benefits of increased production have not been so widely seen in Africa.

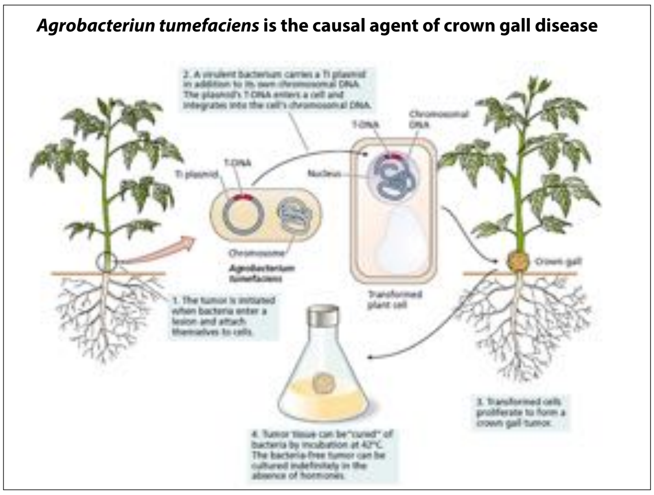


32

Until the early 1980s, the generic modification of crops require the introduction of new genes through sexual crossing and refinement of traits through breeding. Specialised breeding techniques can allow access to gene pools outside of the same species - but access is confined to closely related plants. The advent of techniques to create transgenic plants allows access to effectively any engineered DNA construct and unconstrained modification of plant genomes. This breakthrough came in 1983 with the publication of the first Agrobacterium-mediated plant transformation papers from three groups.

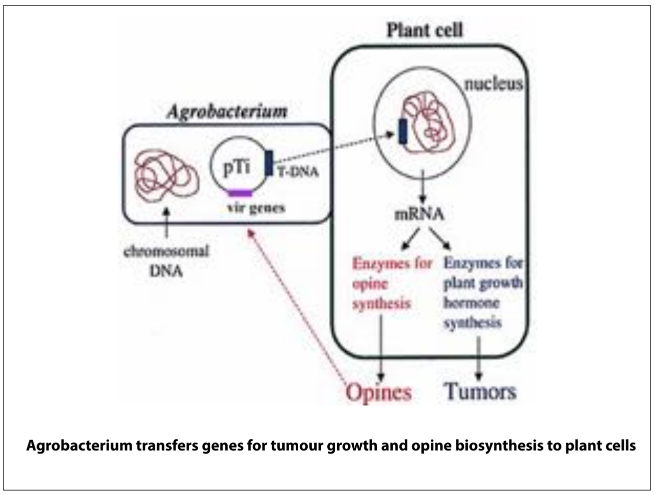
33

Agrobacterium tumefaciens is capable of binding to plant cells, forming a conjugation complex and transferring a specific and delimited segment of DNA. Here shown in an electron micrograph.



34

In a normal infection, conjugation of a bacterium to a susceptible plant is followed by replicative transfer of a specific segment of DNA called the T-DNA (shown in red) - from a region of a Ti plasmid into a recipient plant cell. The transformed cells are then programmed to proliferate.



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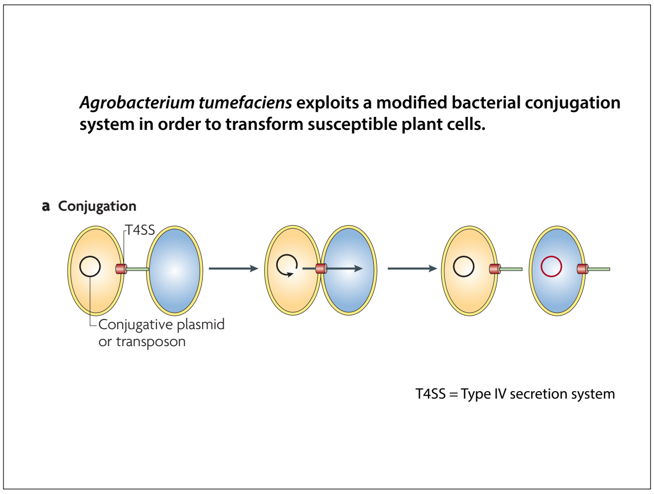
The transferred T-DNA includes genes that encode enzymes for synthesis of plant growth hormones. The ectopic production of growth factors results in unregulated plant cell growth and formation of tumours. In addition, the T-DNA encodes enzymes for production of highly unusual metabolites, called opines. Growing tumours produce opines, which can diffuse into the surrounding soil.

Different races of *Agrobacterium* employ different chemical species of opine (e.g. nopaline, octopine, agropine, etc.) Bacteria of the same type encode genes for transport and metabolism of the corresponding opine on the Ti plasmid. (The Ti plasmid encodes both genes for bacterial expression and those destined for transfer to, and expression in the plant). In nature, Agrobacteria use plant transformation to create local sources of opine, as nutrient that they alone can consume.



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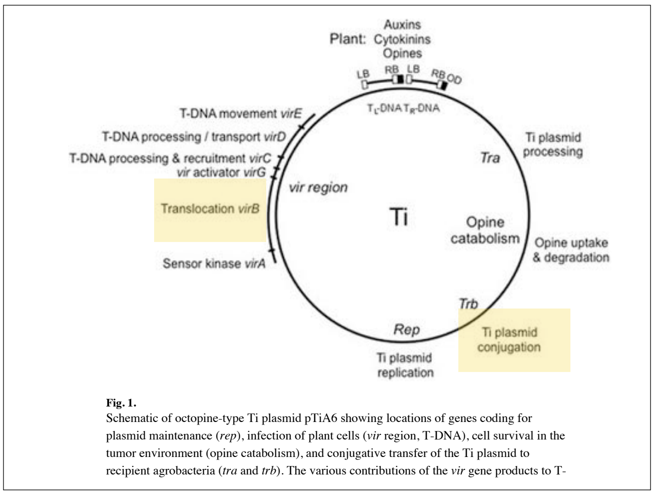
Agrobacterium tumefaciens exploits a modified bacterial conjugation system (a multi-gene Type IV secretion system T4SS) in order to transform susceptible plant cells.



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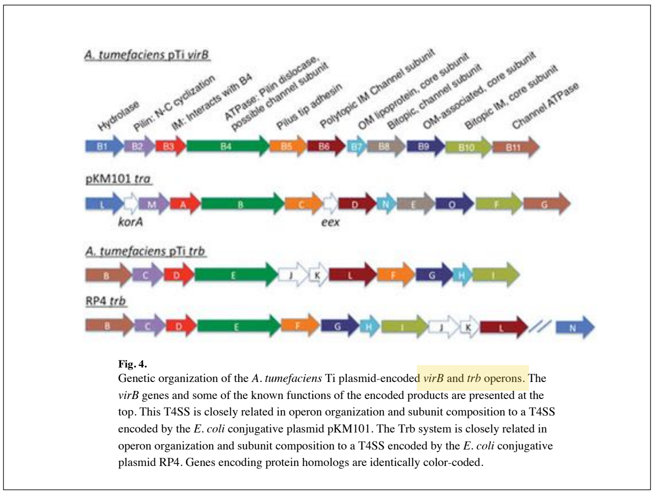
The large Ti (mega-)plasmids have a modular structure, with grouped sets of genes that play distinct functional roles. First, they encode two different Type IV secretion systems, Trb for Ti plasmid transfer and virA for T-DNA transfer (yellow).



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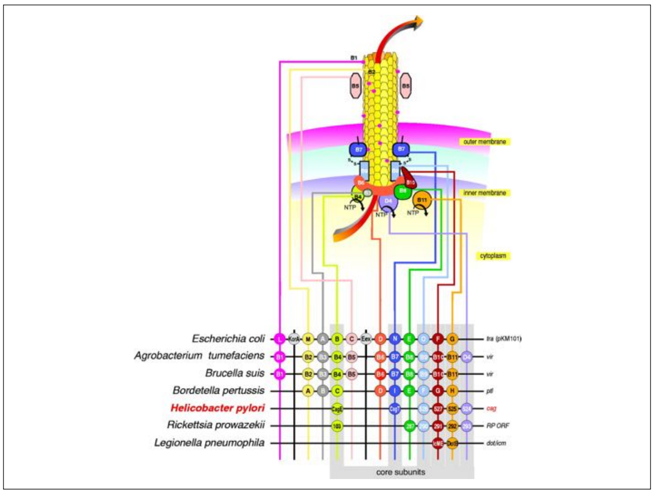
The Type IV secretion systems are encoded in multigene operons, which are highly homologous. Similar machinery is found for T4SS involved in conjugative transfer between bacteria, and between bacterium and plant.



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Diagrammatic representation of the Type IV secretion system and conserved protein subunits found conserved among different bacterial species.

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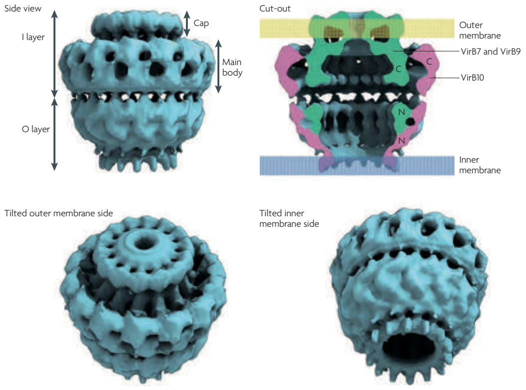


Figure 4 | **Structure of a type IV secretion core complex.** The core complex[53] is composed of TraN (a VirB7 homologue), TraO (a VirB9 homologue) and TraF (a VirB10 homologue), which are encoded by the *Escherichia coli* conjugative plasmid pKM101. This structure was obtained using cryo-electron microscopy and is viewed from the side (upper left panel), tilted towards the outer membrane side (lower left panel) and tilted towards the inner membrane side (lower right panel). The cut-out view (upper right panel) details the proposed transmembrane regions and the localization of the VirB7, VirB9 and VirB10 homologues within the structure. C, carboxy-terminal domain; N, amino-terminal domain.

Cryo-electronmicroscopy reconstruction of the secretion complex core

41

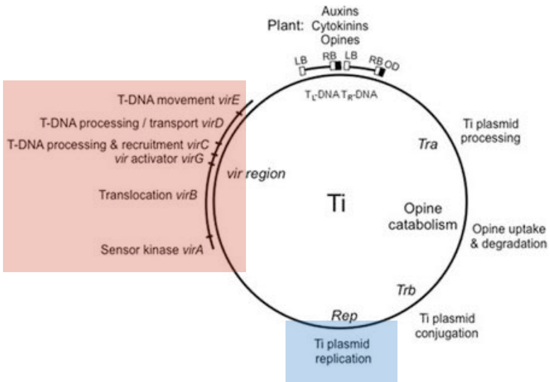
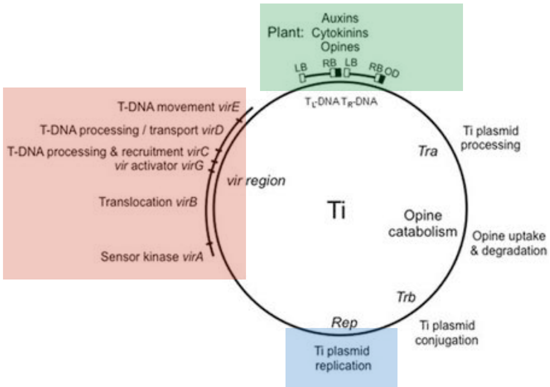


Fig. 1.
Schematic of octopine-type Ti plasmid pTiA6 showing locations of genes coding for plasmid maintenance (*rep*), infection of plant cells (*vir* region, T-DNA), cell survival in the tumor environment (opine catabolism), and conjugative transfer of the Ti plasmid to recipient agrobacteria (*tra* and *trb*). The various contributions of the *vir* gene products to T-

Second, the Ti plasmid encodes proteins and origin required for plasmid replication in the bacterial host (blue).

Third, the entire virulence region (vir) encodes proteins required for sensing wounded plant tissues, activating the vir operon, processing and transfer of the T-DNA in the recipient plant cell (including the Type IV secretion system). Shown in red.

42



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Fourth, the Ti plasmid contains one or more T-DNA (transfer DNA) regions (shown in green). Each is flanked by a specific 25 base-pair sequence, and these boundaries are termed left and right borders.

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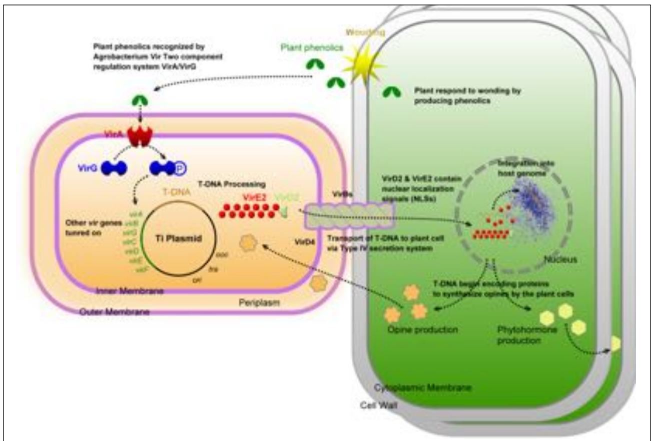
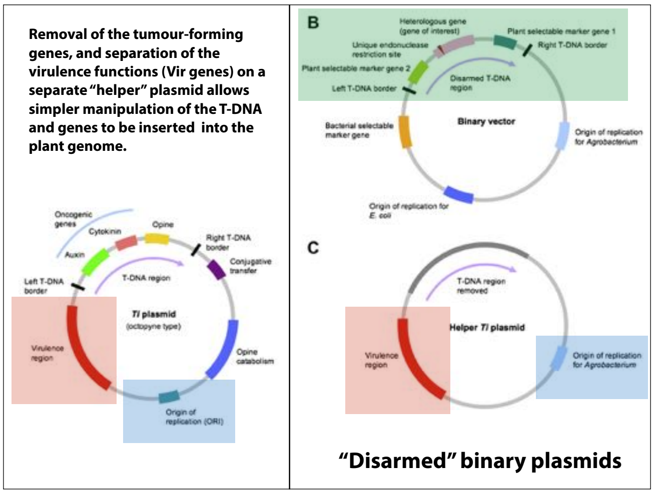


Agrobacterium transformation of plant cells is mediated by intercellular signalling, attachment, virulence protein catalysed DNA transfer to the nucleus and genome integration.

Diagrammatic representation of the induction process, mobilisation and transfer of the T-DNA segment into the recipient plant cell, and integration of the DNA into the host plant genome. Wounded plant cells liberate phenolic compounds, which are sensed by bacterial membrane receptors. These activate the signal transduction pathway and result in transcriptional activation of the virulence operon. Vir genes are responsible for recognition of the T-DNA segment at 25 base pair recognition sequences. Single-stranded DNA nicks trigger a specific replicative transfer of the T-DNA into the plant cell via the Type IV secretion system as a protein-coated single-stranded DNA complex. The defined T-DNA sequence is integrated randomly into the plant genome as a double-stranded segment. The T-DNA segment contains genes with plant control sequences. Once integrated, their expression gives rise to plant hormone and opine production.



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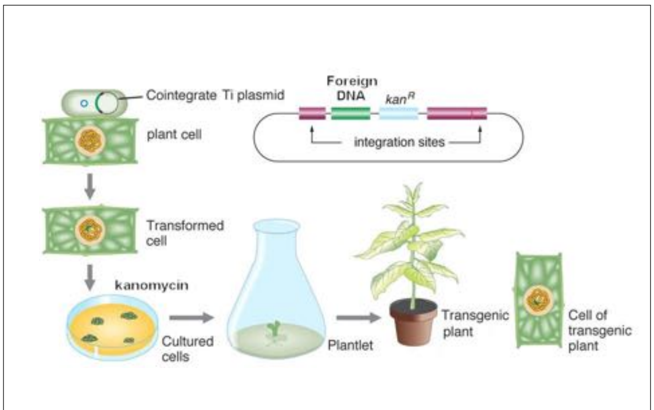
The native Ti plasmid can be disassembled according to its modular nature, and the functions required for tumourigenesis and opine production removed.

The functions required for DNA transfer to the plant can be maintained on a large disarmed plasmid. This is termed a helper plasmid. The gene functions required for DNA transfer can work *in trans* for a second smaller plasmid containing a customised T-DNA segment, along with compatible replication machinery and bacterial selection marker. This forms a binary plasmid system.

This allows simple engineering of new genes on a shuttle plasmid that allows Agrobacterium-meditated transformation of plants.



Summary of Agrobacterium mediated gene transfer and plant regeneration

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Plant transformation with a disarmed binary plasmid requires (i) co-cultivation of plant material with an engineered Agrobacterium strain, (ii) curing of the Agrobacterium by (microbial) antibiotic treatment, (iii) regeneration of plantlets from transformed cells under (plant specific) antibiotic selection. In this example, the engineered T-DNA contains kanamycin. (iv) Rescue of regenerated plants for grow and harvest transgenic seed. At this point transgenic plants can enter a breeding programme.

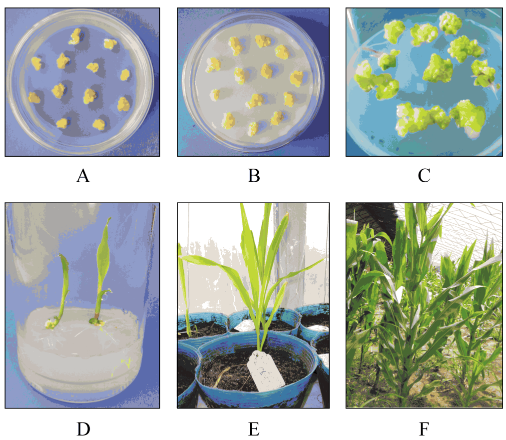

Figure 4 Regeneration of transgenic maize plants

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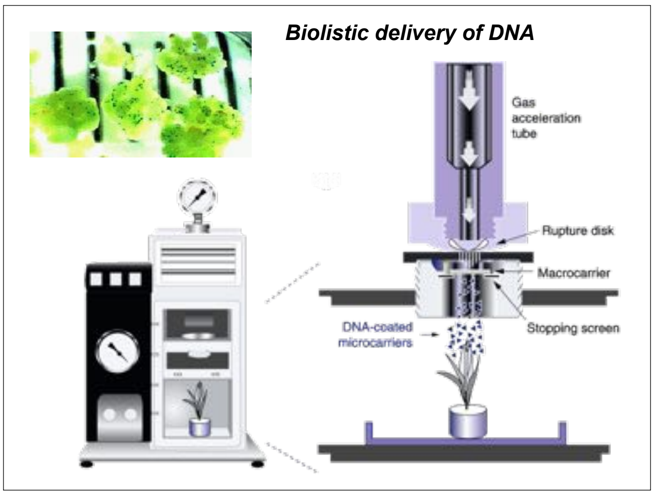
Returning to maize as an example, here are images of transformed and regenerating maize tissues, plantlets and fertile plants.



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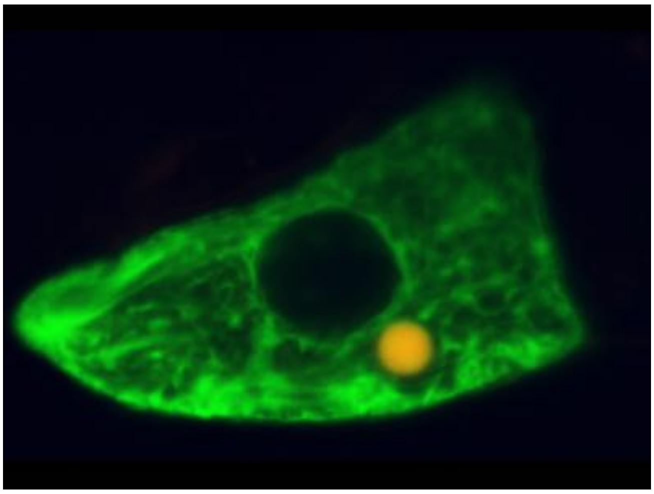
Agrobacterium-mediated transformation is not the only way to produce transgenic plants. High velocity, biolistic delivery of DNA-coated microparticles (usually gold or tungsten) can also be used to produce transgenic plants and algae. This is the method of choice for transformation of organelles.



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A confocal micrograph of a wheat embryogenic cell that has been bombarded with a colloidal gold particle coated with DNA containing an active gene for an ER-localised green fluorescent protein. DNA has been delivered to the cytoplasm of the cell, accumulated in the nucleus (unlabelled in the centre of the cell), and been transcribed. Messenger RNAs have been exported back to the cytoplasm, where they were translated and the green fluorescent protein product accumulated within the endoplasmic reticulum.

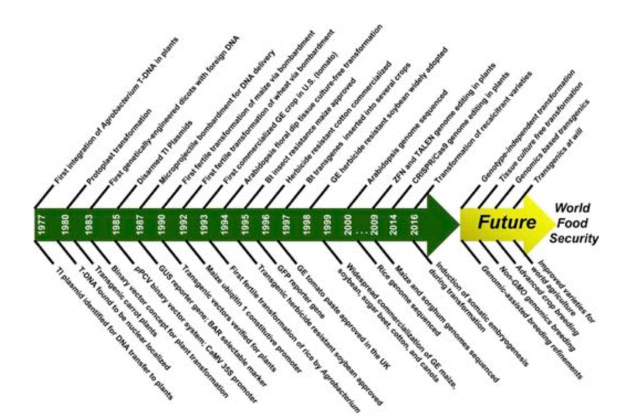

Figure 3. Important Historical Milestones in Plant Transformation.

Since its beginning in 1977, the pace of crop transformation technology development has not been linear. In recent years, the genome editing revolution begs for crop transformation improvements to enable greater food security.

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Time line for recent milestones in transgenic plant work. The next lecture will explore some of these advances in more detail. We will explore gene structure in plants - i.e. how do you successfully build a new plant gene? - and look more closely at what types of genes are used in the commercial world, and how one employs reporter genes to explore the link between genotype and phenotype.